NUTRITIONAL BUFFERING STRATEGIES TO IMPROVE EXERCISE CAPACITY AND PERFORMANCE

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NUTRITIONAL BUFFERING STRATEGIES TO IMPROVE EXERCISE CAPACITY AND PERFORMANCE

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EDITORIAL
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Editorial: Nutritional Buffering Strategies to Improve Exercise Capacity and Performance

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Editorial on the Research Topic

Nutritional Buffering Strategies to Improve Exercise Capacity and Performance

Research has demonstrated that a decline in muscle pH can inhibit contractile function and is a significant contributor within the fatigue paradigm associated with prolonged, high intensity exercise performance. A large body of work has demonstrated the efficacy of nutritional interventions, such as beta-alanine and sodium bicarbonate supplementation, that appear to support endogenous buffering mechanisms tasked with maintaining systemic pH during high rates of anaerobic metabolism, such as prolonged high-intensity exercise (1, 2). Although exogenous buffering supplementation has been studied for close to 100 years (3), numerous questions remain (both applied and mechanistic), which ultimately provided the impetus for this research topic. Collectively, Nutritional Buffering Strategies to Improve Exercise Capacity and Performance provided a consolidated platform for researchers to come together and disseminate novel data that are redefining our understanding and implementation of these nutritional strategies. This brief editorial summarizes and highlights some of these novel findings, beginning with the intracellular buffer beta-alanine.

Rezende et al. performed a systematic review and meta-analysis on studies that measured the muscle carnosine response to beta-alanine supplementation. The analytical model showed that muscle carnosine is relatively stable in skeletal muscle in the absence of supplementation. Human skeletal muscle has a large capacity for muscle carnosine accumulation and effectually all individuals respond to beta-alanine supplementation (albeit with large variability in the magnitude of the response). As further discussed by Perim et al. in their narrative review, commonly used beta-alanine supplementation protocols do not come close to saturating muscle carnosine content, providing scope to optimize supplementation strategies to increase muscle carnosine content. Therein the authors discuss modifiable factors that may better optimize the muscle carnosine response to beta-alanine supplementation including dose and duration of supplementation, supplement formulation, diet, exercise, and co-supplementation with other substances. The dose and duration of supplementation appear to be the main modifiable factors that have a substantial influence on the muscle carnosine response to supplementation, and the authors highlight appropriate recommendations to guide future research.

The importance of supplementation duration is consistent in both reviews (2, 3), as further highlighted by Ribeiro et al. This contribution demonstrated that 3 weeks of beta-alanine supplementation throughout an intense training period before an international

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competition did not attenuate the negative effect that training had on high-intensity intermittent exercise capacity in female footballers. Although it is unclear why exercise capacity was reduced following training, the decline in both aerobic (YoYo Intermittent Recovery Test) and anaerobic (Running Anaerobic Sprint Test & 20-m maximal sprint) capacity suggest that international teams may inadvertently overload their players during these short periods. Whether beta-alanine supplementation may attenuate this decline in capacity is still unknown given the short-duration of the supplementation period likely being sub-optimal. Future research designs might consider starting supplementation earlier to ensure sufficient increases in muscle carnosine prior to the training period.

In the field of extracellular buffering, three original studies investigated the effects of novel sodium bicarbonate supplementation strategies on various aspects of exercise performance. Boegman et al. recruited an impressive 23 world-class rowers to determine whether ingestion timing would impact a 2,000-m rowing time-trial performance. The authors demonstrated that when the time-trial commenced at a time corresponding to an individual rower's peak change in blood buffering capacity after ingesting $0.3\,g{\cdot}kg^{-1}$BM of sodium bicarbonate, their performance was improved when compared to a standardized ingestion timing of 60-min prior to exercise. Although the performance gains were small (less than a 2 s improvement), this finding may be noteworthy given the caliber of athletes included in this study cohort. Another interesting study showed that both a 0.2 and $0.3\,g{\cdot}kg^{-1}$BM dose of sodium bicarbonate may improve outcomes associated with high-intensity interval training (HIIT) even after a standardized 60-min ingestion period Gurton et al. Although both doses resulted in mild-to-moderate gastrointestinal symptoms, the ability to sustain maximal aerobic power was prolonged in a dose-response manner (∼14% for $0.2\,g{\cdot}kg^{-1}$BM & ∼26% for $0.2\,g{\cdot}kg^{-1}$BM) when compared to placebo. Finally, another study demonstrated the possibility that incremental doses of sodium (10, 20, 50 $mmol{\cdot}L^{-1}$) by itself may be ergogenic. These authors observed dose-response improvements in groundstroke performance in nationally-ranked British tennis players Munson et al. Performance improvements were associated with a reduction in ratings of perceived exertion, perception of thirst and gastrointestinal discomfort.

Similar to Gurton's HIIT application Gurton et al., one study also investigated the effects of sodium bicarbonate as a strategy to accelerate recovery between high-intensity exercise bouts Gough et al. Ten minutes following a boxing-specific high-intensity interval running protocol followed by a high-intensity run to exhaustion, seven elite male professional boxers ingested $0.3\,g{\cdot}kg^{-1}$BM of sodium bicarbonate. Participants then completed a boxing-specific punch combination protocol followed by another high-intensity run to exhaustion. Time-to-exhaustion in the second exhaustive exercise bout was greater with sodium bicarbonate compared to placebo (an increase of 164 ± 90 s vs. an increase of 73 ± 78 s) and was accompanied by greater recovery of acid-base balance, including blood pH and bicarbonate. These data suggest that sodium bicarbonate may also be an effective recovery tool for athletes engaged in repeated-bout activities such as boxing and combat sports.

The final study on extracellular buffers showed that the size of the capsules in which sodium bicarbonate is ingested can alter the pharmacokinetic response of blood pH and bicarbonate following supplementation Middlebrook et al. The importance of capsule size and other issues related to pharmacokinetics may have real practical relevance considering the importance of ingestion timing, as highlighted by Boegman et al. Specifically, small capsule sizes led to quicker increases and time-to-peak values of blood bicarbonate, when compared to medium and large capsules. In practice, individuals aiming to increase buffering capacity quickly might want to consider supplementing sodium bicarbonate in small capsules.

In conclusion, this research topic has resulted in a novel collection of articles that have furthered our knowledge in the area of personalized sport and exercise nutrition. We hope that the research included in this topic will act as a potent stimulus for further research in this exciting area and welcome any future topics that may build upon the evidence presented in these papers.

AUTHOR CONTRIBUTIONS

All authors contributed equally and approved the final version.

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REVIEW
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Can the Skeletal Muscle Carnosine Response to Beta-Alanine Supplementation Be Optimized?

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Carnosine is an abundant histidine-containing dipeptide in human skeletal muscle and formed by beta-alanine and L-histidine. It performs various physiological roles during exercise and has attracted strong interest in recent years with numerous investigations focused on increasing its intramuscular content to optimize its potential ergogenic benefits. Oral beta-alanine ingestion increases muscle carnosine content although large variation in response to supplementation exists and the amount of ingested beta-alanine converted into muscle carnosine appears to be low. Understanding of carnosine and beta-alanine metabolism and the factors that influence muscle carnosine synthesis with supplementation may provide insight into how beta-alanine supplementation may be optimized. Herein we discuss modifiable factors that may further enhance the increase of muscle carnosine in response to beta-alanine supplementation including, (i) dose; (ii) duration; (iii) beta-alanine formulation; (iv) dietary influences; (v) exercise; and (vi) co-supplementation with other substances. The aim of this narrative review is to outline the processes involved in muscle carnosine metabolism, discuss theoretical and mechanistic modifiable factors which may optimize the muscle carnosine response to beta-alanine supplementation and to make recommendations to guide future research.

Keywords: optimizing supplementation, muscle carnosine content, metabolism, buffering, modifying factors

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INTRODUCTION

Carnosine is a histidine-containing dipeptide formed by beta-alanine (BA) and L-histidine that is abundant in human skeletal muscle (1). It performs a number of roles which may impact exercise such as antioxidant activity (2–5), antiglycation effects (6), enhanced calcium sensitivity (7, 8), and hydrogen ion (H^+) buffering (9–11). In particular, the biological function of carnosine as a muscle buffer makes it an interesting compound for high-intensity exercise since performance during this type of activity may be influenced by H^+ accumulation and can be improved by increasing buffering capacity (12). Accordingly, carnosine continues to attract interest due to its potential ergogenic benefits, with numerous investigations specifically focused on increasing its intramuscular content to optimize performance (13).

Beta-alanine is a non-proteogenic amino acid and the limiting factor for carnosine formation in the skeletal muscle (1). Chronic supplementation of BA between 4 and 24 weeks appears to

be safe (14, 15) and can increase skeletal muscle carnosine content by up to 200% (16). Strong evidence supports the ergogenic role of BA supplementation for high-intensity exercise with meta-analytical data demonstrating its efficacy, particular during exercise 30 s to 10 min in duration (13). Despite growing evidence supporting the use of BA to enhance exercise performance, the individual response of muscle carnosine to supplementation is highly variable (16) and the amount of ingested BA converted into muscle carnosine appears to be low (17–19). Little is known about modifiable factors that may potentially influence the response of muscle carnosine content to BA supplementation. These factors include dose, duration, meal co-ingestion, co-supplementation with other compounds, and exercise. Enhanced understanding of these factors is of interest to athletes, support staff and researchers, as greater increases in muscle carnosine are associated with greater improvements in exercise capacity (16, 20). The aim of this narrative review is to outline the processes involved in muscle carnosine metabolism, discuss theoretical and mechanistic modifiable factors which may optimize the muscle carnosine response to BA supplementation, and to make recommendations to guide future research in this area.

MUSCLE CARNOSINE METABOLISM

Carnosine homeostasis is dependent on its synthesis from, and degradation to, its constituent amino acids. Carnosine is synthesized from BA and L-histidine in a reaction catalyzed by the non-specific enzyme carnosine synthase (*CARNS*), an enzyme located in skeletal muscle (21). Importantly, beta-alanine has a high affinity (K_m, 1.0–2.3 mM) for carnosine synthase (22) along with a low muscle content (∼0.2 mmol·kg^{-1}ww) (23); histidine, on the other hand, is found in high concentration in muscle (∼0.4 mmol·kg^{-1}ww) (24) but has a low K_m (16.8 μM) for carnosine synthase (25). These data indicate that BA is the rate-limiting amino acid to muscle carnosine synthesis, a finding that is corroborated by supplementation studies that show that BA alone is similarly effective at increasing muscle carnosine content, than an equivalent dose of BA delivered in carnosine (which comprises both BA and histidine) (1).

Carnosinase is a hydrolytic enzyme found in serum and tissue (26) that actively degrades carnosine into its constituent amino acids (27). Serum carnosinase (also known as carnosinase-1) is highly specific for carnosine while carnosinase found in tissue (also known as carnosine-2) has a broader substrate specificity (28). Despite its presence in skeletal muscle as a cytosolic non-specific dipeptidase, carnosinase-2 functions optimally at pH 9.5 (26, 29) which is far in excess of the pH 7.4 typically encountered in human muscle meaning it has little influence in muscle. The presence of carnosinase in the gastrointestinal tract (30) means that some ingested carnosine, or histidine containing dipeptide analogs such as anserine or balenine (28), may be hydrolysed to BA and histidine before reaching the blood stream. Nonetheless, most carnosine will reach the blood stream where carnosinase-1 is highly present and active in humans, meaning that the carnosine that reaches the bloodstream is immediately hydrolysed into BA and histidine. Indeed, very little carnosine is found in human blood (31) and carnosinase activity is considered the main determinant of circulating carnosine levels following dietary carnosine ingestion (32). The constituent amino acids can then be transported to the muscle (**Figure 1**).

The uptake of BA into muscle is primarily mediated by *TauT*, a specific β-amino acid transport protein also responsible for the uptake of taurine into muscle that is dependent upon stoichiometric concentrations of both Na^+ and Cl^- in a 2:1:1 (Na^+:Cl^-:β-amino acid) ratio (33). The BA transporter *TauT* into muscle cells has a K_m of 40 μM (34) which is relatively high compared to the <0.5 μM of BA typically found in blood (1) meaning that circulating levels must be increased to augment transport into muscle. Another transporter, *PAT1*, also transports BA into muscle although its contribution appears minimal compared to *TauT* (35). For these reasons, it is commonly accepted that the transport of BA into muscle is predominantly determined by the *TauT* transporter. While several non-specific peptide transporters (*PEPT1*, *PEPT2*, *PHT1*, *PHT2*) exist which can transport carnosine and its methylated analogs, only *PHT1* is found in abundance in human skeletal muscle and PEPT2 to a lesser extent (35).

Endogenous production of BA is low and occurs primarily inside the liver through the degradation of uracil (36). For this reason, dietary sources of histidine containing dipeptides (e.g., carnosine, anserine, balenine) such as meat, fish, and poultry [e.g., 200 g chicken breast contains ∼800 mg of BA (1)] may be a determinant of muscle carnosine content (37). In support of this, vegetarians, whose only source of BA is endogenous production, have significantly lower muscle carnosine content compared to their omnivorous counterparts (38). However, omnivores who were put on a 6-month vegetarian diet did not reduce their muscle carnosine stores, suggesting carnosine homeostasis is tightly regulated and not entirely dependent on dietary intake (39). Nonetheless, it is unquestionable that BA intakes in excess of dietary intake are required to elicit significant carnosine increases (39), meaning supplementation with BA is the most effective and practical means by which to increase muscle carnosine content.

THE INFLUENCE OF BETA-ALANINE SUPPLEMENTATION ON MUSCLE CARNOSINE METABOLISM

The first study to show that BA could increase the intramuscular carnosine pool measured a +40–60% increase in carnosine content of the *m. vastus lateralis* (1), as measured by high-performance liquid chromatography (HPLC) of muscle biopsy samples. Numerous studies have corroborated these findings using HPLC (16, 20, 23, 24, 40) and proton magnetic resonance spectroscopy (^{1}H-MRS) (41–46). Almost all individuals across these independent studies showed increases in muscle carnosine following a period of BA supplementation although there is a

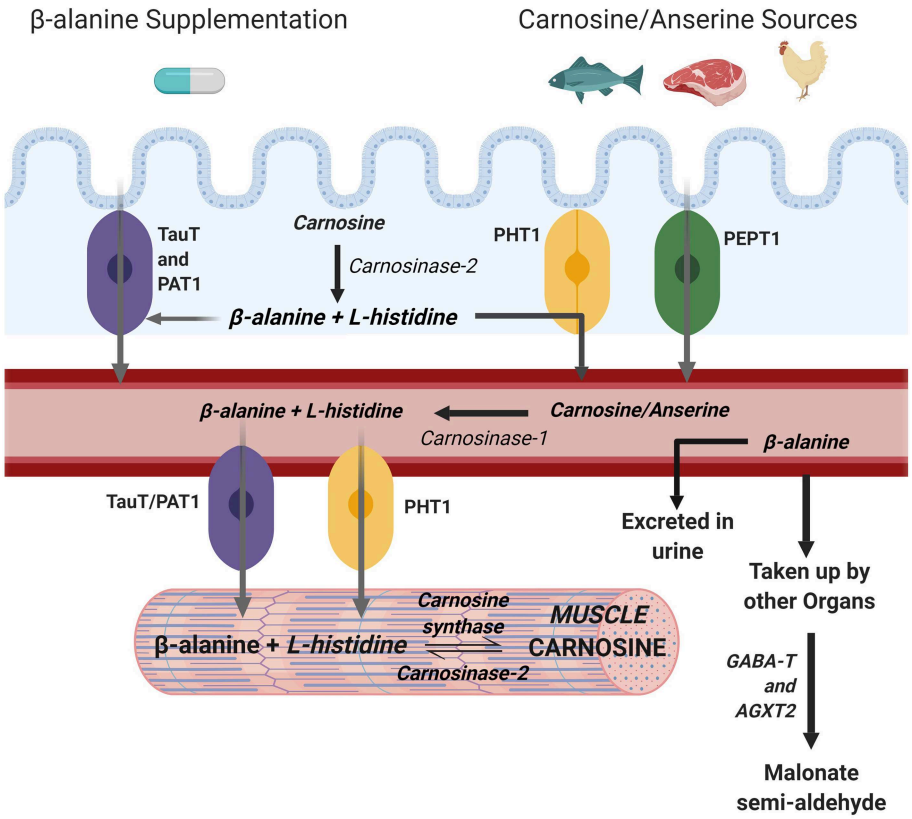

FIGURE 1 | Metabolism of muscle carnosine. Created with BioRender.

large variability in the magnitude of this response, both between and within studies. Data from our laboratory has shown maximal absolute increases of between +17 to +41 mmol·kg^{-1}dm (+59 to +200%), highlighting the range of change between individuals. Variable responses are likely due to a combination of modifiable (i.e., dose, duration, co-supplementation, etc.) and non-modifiable (i.e., age, gender, disease) factors, although herein we will focus on the modifiable factors through which individuals might optimize muscle carnosine loading. Surprisingly, despite consistent and large increases in different muscle groups with BA supplementation, evidence suggests actual incorporation of BA into muscle carnosine is low. The amount of BA ingested that is converted into muscle carnosine is only about 3–6% (17–19), meaning that upwards of 90% of ingested BA is directed toward other physiological outcomes, which may include transamination and oxidation (47), while small amounts (∼3%) are also lost via the urine (1). Understanding of the primary mechanisms by which increased BA availability increases muscle carnosine content is an essential step to see if its incorporation into muscle can optimized, while determination of the importance of these alternative pathways through which the majority of BA is metabolized may provide further scope for investigation.

It appears reasonable to expect that any changes in muscle carnosine content would be paralleled by changes in the proteins involved in its metabolism. Everaert et al. (35) showed upregulation of several genes encoding proteins and enzymes involved in carnosine homeostasis in response to 8 weeks of BA supplementation in mice. Specifically, gene expression for the enzymes relating to BA transport into muscle (*TauT*), synthesis of muscle carnosine (*CARNS*), and the deamination of BA (*ABAT*) increased expression, suggesting an important role for these proteins in increasing muscle carnosine content. The only study to measure changes in gene expression with BA supplementation in humans showed a chronic downregulation of *TauT* during 24-weeks of supplementation at 6.4 g·day^{-1}, but no change in any other genes (16). It currently remains unclear why these two studies showed such contrasting results in gene expression following BA supplementation, particularly in reference to *TauT*. A key difference may be the timing of muscle sampling as it is unclear when dissection of the mice was performed relative to the last BA dose (35) while the human samples were always taken at least 4 h after the last ingested dose of BA. The time course response of carnosine-related gene expression following acute BA supplementation should be determined to further understand these findings since a single

end-point biopsy following an intervention can influence the inferences made (48).

Muscle carnosine loading is most pronounced during the initial weeks of supplementation, after which increases in muscle carnosine content appear to slow. This is certainly true of the first vs. subsequent 12 days of supplementation (40), and the first 4 weeks compared to the remaining 20 weeks of supplementation (16). This slowing may be due to a decreased transport of BA into muscle, suggested by the downregulation of *TauT* gene expression (16). Despite this and as reported previously, intramuscular carnosine levels follow a progressive increase as long as supplementation continues whereby reported intramuscular carnosine content was greater after 20 and 24 weeks of supplementation when compared to 8 weeks of supplementation (16). In fact, several individuals showed substantial increases ($> +6$ mmol·kg^{-1}dm) in the final 4 weeks of supplementation and it is likely that further increases in carnosine would have occurred if supplementation had continued. It seems possible, therefore, that *TauT* downregulation may attenuate the rate of carnosine synthesis in response to continued supplementation, but it does not block it completely. Further work should determine the true contribution of *TauT* to muscle carnosine increases with BA supplementation.

Beta-alanine can be transaminated into malonate semi-aldehyde by the enzymes GABA-T and AGXT2 for further metabolism within the citric acid cycle; these enzymes are highly expressed in kidney and liver of mice, but exhibit low expression in muscle (47). Low dietary intake of BA (0.1% BA in drinking water) in mice did not increase circulating BA or muscle histidine-containing dipeptide content (47), although when this low BA dose was provided alongside simultaneous inhibition of these BA transaminating enzymes, this led to increased circulating BA, and histidine-containing dipeptide content was increased in muscle and heart. These data suggest that low doses of BA may be entirely transaminated by highly active transaminating enzymes, leading to minimal to no changes in circulating BA or muscle histidine-containing dipeptide content, but should saturation of these enzymes occur, significant increases in the tissue concentrations of histidine-containing dipeptides can occur. The authors suggest that saturation of these enzymes is unlikely to occur with normal human dietary patterns, perhaps explaining the relative stability of muscle carnosine over time (42). It is possible however, that acute dietary ingestion via meat or fish may be sufficient to saturate these enzymes, since omnivores have higher muscle carnosine content than vegetarians (38). Certainly, it appears more than likely that under conditions of excess BA availability, such as supplementation, enzyme saturation occurs leading to increased circulating levels of BA and eventual uptake into skeletal muscle resulting in elevated intramuscular carnosine content. Since doses of BA as low as 1.6 g·day^{-1} lead to increases in muscle carnosine content (46), the likelihood that BA supplementation at these doses do indeed saturate these transaminating enzymes is high. The relevance of these alternate pathways of BA transamination may be an avenue of interest for further investigation.

It is currently unknown to what extent the acute plasma BA response to supplementation is related to chronic changes of carnosine in muscle when BA is ingested over an extended period. It could be hypothesized that greater increases in circulating BA may be due to lower transamination and, thus, may result in larger increases in muscle carnosine content. Supporting this, the carnosine and anserine concentration of murine skeletal and heart muscle appears dependent upon the circulating availability of BA (47). In humans, Stautemas et al. (49) showed a large inter-individual variability in the pharmacokinetic plasma BA profile following an acute absolute 1,400 mg dose of BA. Importantly, the high variability in plasma BA was not reduced after a dose relative to body mass. It is known that the time course plasma profile following an acute dose of BA appears stable throughout a period of chronic supplementation (1). Unfortunately, neither of these studies related chronic changes in muscle carnosine to the acute plasma BA profile, which may provide answers as to the importance of this initial acute plasma response to predict chronic changes and may direct future research in the area.

MODIFIABLE FACTORS INFLUENCING THE INCREASES IN MUSCLE CARNOSINE CONTENT WITH BETA-ALANINE SUPPLEMENTATION

Dose and Duration

The largest contributing factors to changes in muscle carnosine content appear to be the daily dose provided and the duration of supplementation. Doubling of the BA dose (12 vs. 6 g·day^{-1}) halves the time taken to reach the same increases in the *m. vastus lateralis* (50). Similarly, Stellingwerff et al. (46) showed two-fold greater increases in carnosine of the *tibilias anterior* and *gastrocnemius* at a higher dose of 3.2 compared to 1.6 g·day^{-1} of BA for 4 weeks. Moreover, when supplementation was continued at 1.6 g·day^{-1} in both groups up to 8 weeks, muscle carnosine also continued to increase. Thus, there is strong evidence to show that a higher dose and/or longer supplementation period leads to greater accumulation of muscle carnosine. The muscle carnosine response to supplementation was initially proposed to be linearly related to the total amount of BA consumed (51). However, although doubling the dose appears to double the increases in muscle carnosine content during the first 2–4 weeks of supplementation (46, 50), a higher dose taken for a longer period (6.4 g·day^{-1} for 24 weeks) shows a slowing over time (16), suggesting this response is not linear.

Spelnikov and Harris (52) proposed a mathematical model describing the kinetics of carnosine accumulation in human skeletal muscle based on its rate of synthesis and decay. Using existing data, the model estimates that the rate of synthesis of carnosine in human skeletal muscle is constant over time for any given dose of BA, but that the rate of decay will change according to first-order kinetics (52). The washout of muscle carnosine has been shown to occur over several weeks to months before returning to similar pre-supplementation levels when supplementation ceases (42, 46), and could occur due to a

number of reasons including transmembrane leakage and the formation of adducts with carbonyl groups, reactive oxygen species and reactive nitrogen species (5, 28). Based upon these parameters, the time course model of muscle carnosine changes predicts that with any BA dose, saturation for that specific dose will occur over time with continual supplementation. It must be noted that this model is currently speculative and the dose that will cause absolute saturation of carnosine in muscle is unknown; there are no known reports of muscle carnosine saturation in humans. Although the model predicts that a certain level of saturation will occur according to the continuation of supplementation at any specific dose, the first weeks of supplementation appear most susceptible to increases in muscle carnosine content (16) and thus the period most likely to be amenable to optimisation in BA supplementation.

Beta-Alanine Formulation

Current recommendations for beta-alanine ingestion is for it to be taken in staggered doses of 800–1,600 mg every 3–4 h over the day in order to reduce the incidence and severity of paraesthesia, an uncomfortable tingling sensation on the skin that can last up to an hour (1). Although the exact cause of paresthesia is unknown, it is thought to be due to BA activated strychnine-sensitive glycine receptor sites, associated with glutamate sensitive N-methyl-D-aspartate receptors in the brain and central nervous system (53) and the mas-related gene family of G protein coupled receptors, which are triggered by interactions with BA (54). Given that the development of paresthesia is closely related to the time-to-peak beta-alanine concentration in blood following ingestion (1), sustained-release BA formulations have been developed to avoid this side-effect. Such sustained-release formulations directly reduce the symptoms of paresthesia and allow greater single tolerable doses of BA, which in turn will allow larger daily doses. This can lead to greater increases in muscle carnosine in the initial period of supplementation due to higher daily doses (24, 50). In support of this, symptoms of paresthesia while ingesting individual doses of 4 g of BA in sustained-release form were not different from those experienced with 2 g doses (50), meaning greater daily doses could be ingested without further discomfort leading to larger gains in muscle carnosine.

A study by Decombaz et al. (55) showed that ingestion of 1.6 g of BA in slow-release tablets resulted in slower absorption kinetics and improved whole body retention of BA, as measured by urinary excretion of BA, compared to the same dose in aqueous solution. Greater retention of BA suggests that supplementation in a sustained-release format might lead to greater increases in muscle carnosine compared to an instant release (e.g., powder) formulation, although the authors did not measure muscle carnosine in this study. Stegen et al. (19) did not show any differences in muscle carnosine increases in the *m. gastrocnemius* and *m. soleus* between individuals supplementing with 4.8 g·day^{-1} powder or sustained-released BA for 5 weeks. Varanoske et al. (24) compared sustained-release and rapid-release formulations of BA, providing volunteers with 6 g·day^{-1} for 28 days. Muscle carnosine content was significantly increased in the group consuming the sustained-release formulation while, perhaps surprisingly, no significant changes were shown with rapid-release supplementation despite a ∼38% increase. However, the ∼16% difference in elevation of muscle carnosine between the two formulations did not reach statistical significance, perhaps due to the small sample size or the short supplementation period. In fact, forward projecting the increases in muscle carnosine using a mathematical model (52) suggested that large differences would be found between formulations within 100 days of supplementation. However, these data are highly speculative and can only be proven with further research. As it stands, there is some evidence to suggest that supplementation with slow-release BA may enhance muscle carnosine increases relative to an instant-release formulation although this is more likely due to an increased tolerance allowing greater daily doses without the incidence of uncomfortable side-effects. More long-term studies are warranted to evaluate whether the same daily dose in different formulations leads to distinct increases in muscle carnosine content.

Dietary Influences

The timing of ingestion is considered an important factor which may affect the efficacy of many dietary supplements (56, 57). Since BA is ingested at several timepoints throughout the day, it could be important to determine whether the timing of supplementation may influence the subsequent increased in muscle carnosine, particularly around meals and training. It has been suggested that co-ingestion of BA with carbohydrates or a carbohydrate-rich meal may lead to greater muscle carnosine increases than ingesting BA between meals (19) because the carbohydrate-mediated release of insulin upregulates the activity and content of the sarcolemmal Na^+/K^+-ATPase pumps (58, 59). Since the BA transporter *TauT* is dependent on sodium and chloride co-transport (34), muscle BA uptake and subsequently carnosine synthesis may be increased due to the action of insulin. In support of this theory, it is well-established that creatine uptake into muscle, which is also sodium-dependent, can be heightened when supplementation occurs alongside the intake of high glycaemic index carbohydrates (60, 61).

To date, only one study has investigated the potential influence of insulin on muscle carnosine increases with BA supplementation using a two-part investigation (19). Firstly, acute determination of whole-body BA retention showed no difference when BA was ingested in a fasted state or when co-ingested with two energy-rich carbohydrate bars. In Part B, participants ingested BA at 3.2 g·day^{-1} for 6–7 weeks, separated into two groups who were required to ingest the supplement with (co-ingestion) meals or between meals. Meal co-ingestion enhanced muscle carnosine loading in the soleus muscle, but this result was not mirrored in the gastrocnemius. Several mechanisms exists that could explain the difference in muscle carnosine loading between soleus and gastrocnemius, namely, increased insulin sensitivity in the soleus (62) and a preferential insulin-induced translocation of Na^+/K^+-ATPase subunits in oxidative fibers (e.g., the soleus) over glycolytic fibers (e.g., the gastrocnemius). Intramuscular Na^+-K^+-pump

activity can also be stimulated by caffeine (58), meaning that co-supplementation of caffeine and BA may enhance muscle carnosine loading through this mechanism as well. While data surrounding dietary confounders, such as meal co-ingestion or caffeine co-supplementation, on the *TauT*-mediated transport into muscle and how this may impact intramuscular carnosine concentrations is lacking, more research in this area may allow development of supplementation strategies to enhance carnosine uptake into the skeletal muscle.

Influence of Exercise

Increases in muscle carnosine content have been hypothesized to be an adaptation to long-term high-intensity training as demonstrated by higher values in bodybuilders (63) and trained sprinters (64). It remains unclear whether this is due to genetic predisposition, an adaptative response to the training stimulus, or secondary to differences in muscle fiber type composition. Certainly, a greater number of type II muscle fibers are shown in resistance and sprint-trained individuals while muscle carnosine has a higher content in type II glycolytic fibers compared to type I oxidative fibers (20, 65). These data have also been attributed to a greater increase of dietary BA (via increased meat intake) or to chronic steroid use in these populations [the anabolic effect of androgens may play a role in muscle carnosine metabolism (35, 66)], although the true effect of these factors remains unclear. Nonetheless, chronic training is often cited as a determinant of increased muscle carnosine content (37).

Despite cross-sectional data suggesting an adaptive role of muscle carnosine in response to training, most short-term (4–16 weeks) exercise training protocols have, however, failed to modify intramuscular carnosine content (65–69). Nevertheless, a recent study demonstrated that, independent of BA supplementation, 12-weeks of high-intensity interval training in vegetarians can increase muscle carnosine content in the absence of any dietary BA intake (70). This indicates that an increase in muscle carnosine synthesis occurred despite no ingestion of BA, meaning there may have been an increase in endogenous BA production, although this was not measured. The major part of these increases was attributed to an increase in muscle carnosine metabolism, although it is unclear what the mechanisms are since no changes in the expression of genes involved in carnosine metabolism were shown. This may have been due to the timing of muscle sampling relative to the training sessions; gene expression was determined from muscle biopsies taken at one timepoint 72 to 96 h following training. The possibility that changes in gene expression occurred at different time points following exercise cannot be excluded while replication of these data in omnivorous individuals is also warranted.

Despite clear evidence that exercise can influence muscle carnosine homeostasis (70), no study to date has shown significantly enhanced muscle carnosine loading when BA supplementation was performed in conjunction with a specific training program (45, 65, 69, 71). The reasons for these findings are unclear since none of these studies measured changes in the enzymes, proteins and transporters involved in muscle carnosine regulation. Furthermore, differences in exercise intensity and modality, training duration and dietary habits challenge the ability to isolate why any given individual study showed no combined effect of training and BA supplementation on muscle carnosine increases. Greater increases in muscle carnosine content were shown in trained vs. untrained muscles following 23 days of supplementation at 6.4 $g \cdot day^{-1}$ despite the athletes not being put through a specific training protocol (72). Kayakers showed more pronounced gains in muscle carnosine in the deltoid muscle compared to the soleus and gastrocnemius, whereas the reverse pattern was seen in cyclists. Swimmers, whose exercise task requires both upper and lower-body training, had significantly higher increases in carnosine in both the deltoid and gastrocnemius compared with non-athletes. These results imply a role of training on muscle carnosine metabolism, although a lack of gene or protein measurements hinders mechanistic interpretation of these findings. The authors suggest an increased delivery of BA to the working muscle cells or a possible contraction-induced stimulation of the BA transporters may have contributed to these differences, but data to support or refute this hypothesis is currently unavailable. Overall, little is currently known on how exercise may influence muscle carnosine metabolism. Consequently, more mechanistic studies are required to determine the effects of both an acute exercise bout and chronic training on the major regulators of carnosine content. This will provide information as to whether there is any physiological relevance to ingesting BA at specific timepoints relative to exercise training.

Co-supplementation of Histidine With Beta-Alanine

Carnosine is formed by BA with L-histidine and is therefore dependent on the availability of both of these amino acids (28). Blancquaert et al. (40) showed a significant depletion of muscle histidine content following 23 days of BA supplementation. The authors speculated that this reduced muscle histidine availability could be the reason for an impaired efficiency of carnosine loading with BA as supplementation is extended over time (16, 40). While co-supplementation of BA and histidine in their study did avoid the depletion of muscle histidine stores, intramuscular carnosine content was unaffected when compared to supplementation with just BA. These results corroborated previous findings of Harris et al. (1) that showed no additive effect of BA and histidine supplementation on muscle carnosine changes. A number of subsequent studies reported no influence of BA supplementation on histidine content, (23, 24, 50), a finding that was since corroborated by meta-analysis of available data (15). Differences in dietary intake may explain some of the differences between these studies since the average American diet generally contains more protein than the typical Belgian diet (23), although this remains highly speculative. Currently, evidence suggests that histidine depletion is not a limiting factor to muscle carnosine synthesis, meaning co-supplementation of BA with histidine (or carnosine supplementation) will not further augment any increases seen in muscle carnosine content. Regardless, future work should continue to explore the effect of one's diet on muscle histidine content and whether prolonged

supplementation with BA at high doses leads to histidine depletion in muscle.

CURRENT RECOMMENDATIONS AND FUTURE INVESTIGATION

Chronic BA supplementation increases the intracellular content of carnosine in skeletal muscle and can subsequently improve sports and exercise performance. Current recommendations (13, 15, 73) based upon the available evidence suggest ingesting 3.2 to 6.4 $g \cdot day^{-1}$ of BA for 4–24 weeks. To avoid the uncomfortable feeling of paraesthesia, it is recommended to fraction daily doses into 0.8 and 1.6 g doses at intervals of 3 to 4 h. Adhering to this supplementation regimen will minimize side-effects and lead to significant gains in muscle carnosine content that can benefit exercise performance. However, since greater increases in muscle carnosine are associated with greater exercise benefits (16), herein we have discussed several factors which may optimize the gains achieved using these current recommendations although further work is necessary to elucidate the most achievable methods by which to optimize the muscle carnosine response to BA supplementation.

Studies have shown that chronic supplementation with BA may lead to upregulation or downregulation of the genes associated with carnosine metabolism, although results are contrasting. It would be of interest to determine what the acute response (i.e., timecourse) of these transporters, proteins and enzymes are following a standard BA dose, and whether these changes reflect or predict the longer-term changes in muscle carnosine content. In particular, evidence suggests that the BA transporter *TauT* likely exerts an important role in the observed changes during supplementation. Several avenues exist to test the importance of this transporter including co-supplementation with taurine [since this downregulates *TauT*; (74)] or caffeine, and *TauT* knockout animal models. Further investigations should also focus on the independent influence of insulin and exercise on muscle carnosine metabolism to determine the exact mechanism(s) by which diet and physical activity may optimize increases in muscle carnosine content with BA supplementation. Investigation into different formulations of BA is needed to determine if sustained-release tablets can enhance muscle carnosine increases with chronic supplementation. It is also crucial to determine whether small gains in muscle carnosine content above those generally shown, induced by manipulation of some of these modifiable factors, do indeed lead to worthwhile improvements in performance.

CONCLUSIONS

Several modifiable factors may optimize the muscle carnosine response to BA supplementation, of which the dose and duration are the strongest known moderators (**Figure 2**). Other factors may optimize increases, particularly during the initial weeks of supplementation, including supplement formulation, ingestion timing in relation to meals and exercise, although stronger evidence to support this is

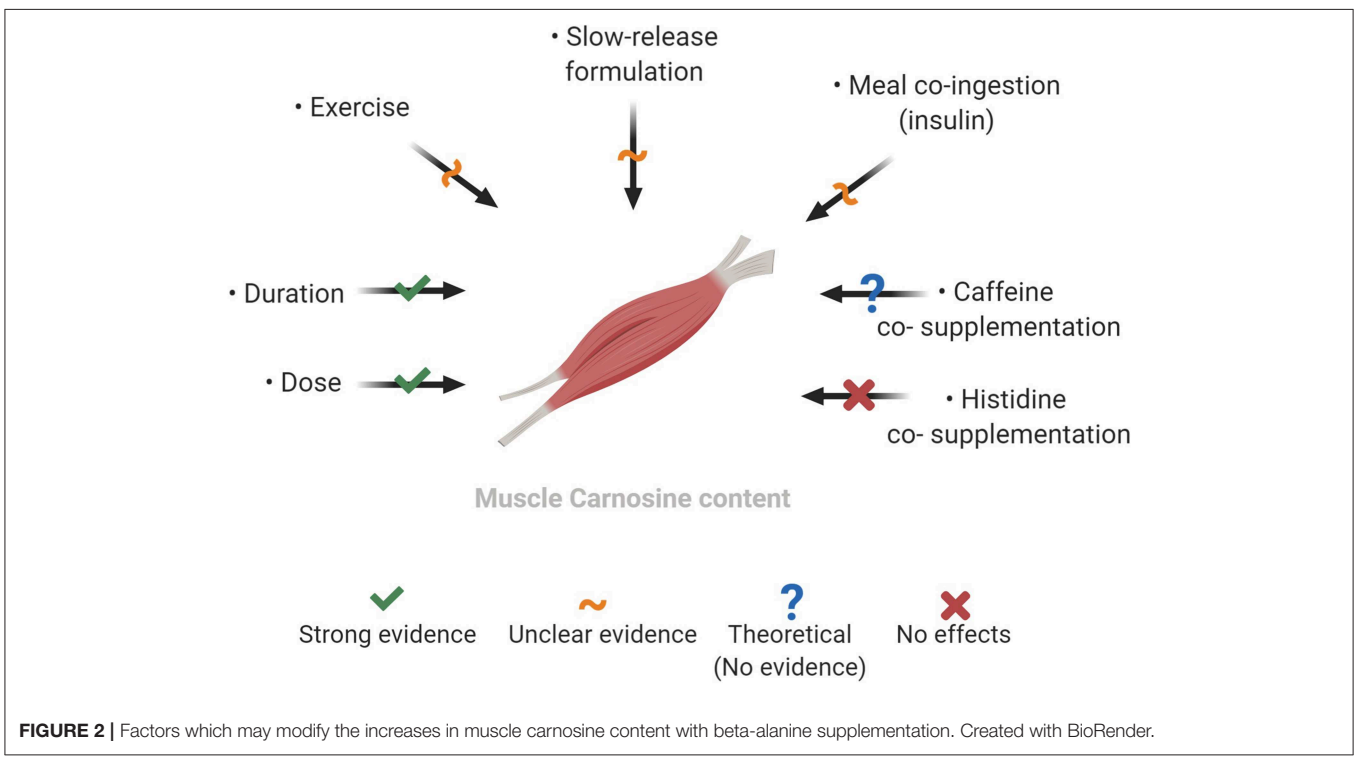

FIGURE 2 | Factors which may modify the increases in muscle carnosine content with beta-alanine supplementation. Created with BioRender.

needed. As it stands, more mechanistic work is necessary to elucidate whether BA supplementation can lead to greater muscle carnosine gains above those shown with current recommendations.

AUTHOR CONTRIBUTIONS

BS and PP are responsible for the conception of the work. PP, FM, FR, GB, NG, and BS are responsible for the initial writing of the manuscript. FM created the figures. CK and ED reviewed and made significant contributions to the manuscript. All authors approved the final version of the manuscript.

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Post-exercise Supplementation of Sodium Bicarbonate Improves Acid Base Balance Recovery and Subsequent High-Intensity Boxing Specific Performance

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The aim of this study was to assess the effects of post-exercise sodium bicarbonate ($NaHCO_3$) ingestion (0.3 g.kg^{-1} body mass) on the recovery of acid-base balance (pH, HCO_3^-, and the SID) and subsequent exercise performance in elite boxers. Seven elite male professional boxers performed an initial bout of exhaustive exercise comprising of a boxing specific high-intensity interval running (HIIR) protocol, followed by a high-intensity run to volitional exhaustion (T_{LIM1}). A 75 min passive recovery then ensued, whereby after 10 min recovery, participants ingested either 0.3 g.kg^{-1} body mass $NaHCO_3$, or 0.1 g.kg^{-1} body mass sodium chloride (PLA). Solutions were taste matched and administered double-blind. Participants then completed a boxing specific punch combination protocol, followed by a second high-intensity run to volitional exhaustion (T_{LIM2}). Both initial bouts of T_{LIM1} were well matched between PLA and $NaHCO_3$ (ICC; $r = 0.94$, $p = 0.002$). The change in performance from T_{LIM1} to T_{LIM2} was greater following $NaHCO_3$ compared to PLA (+164 ± 90 vs. +73 ± 78 sec; $p = 0.02$, CI = 45.1, 428.8, $g = 1.0$). Following ingestion of $NaHCO_3$, pH was greater prior to T_{LIM2} by 0.11 ± 0.02 units (1.4%) ($p < 0.001$, CI = 0.09, 0.13, $g = 3.4$), whilst HCO_3^- was greater by 8.8 ± 1.5 mmol.l^{-1} (26.3%) compared to PLA ($p < 0.001$, CI = 7.3, 10.2, $g = 5.1$). The current study suggests that these significant increases in acid base balance during post-exercise recovery facilitated the improvement in the subsequent bout of exercise. Future research should continue to explore the role of $NaHCO_3$ supplementation as a recovery aid in boxing and other combat sports.

Keywords: buffering, alkalosis, acid base balance, combat sports, recovery, nutrition, training

INTRODUCTION

High levels of glycolytic flux are essential to maintain the required physiological output during combat exercise (1), although a concomitant fall in both muscle and blood pH and bicarbonate ion concentration [HCO_3^-] eventually occurs (2). This is due to the increases in hydrogen ion (H^+) accumulation, which in turn, disturb the state of equilibrium between acidity

and alkalinity of body fluids (i.e., acid base balance). Such an alteration is known as metabolic acidosis and has been associated with fatigue by reducing or impairing the release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum (3), impeding glycolytic enzyme activity (4), and altering the strong ion difference leading to reduced action potentials and muscle excitability (5). The typical daily regimen for a competitive boxer often consists of two sessions comprised of an initial high-intensity intermittent running session followed by a boxing-specific session that mimics the demands of competition, interspersed within a short recovery period (6). Subsequently a large degree of metabolic acidosis is likely evident in the subsequent bout of exercise, therefore mitigation of the deleterious effects between sessions are prudent to investigate.

Pre-exercise ingestion of 0.3 $g.kg^{-1}$ body mass (BM) sodium bicarbonate ($NaHCO_3$) can lead to an approximate increase in pH ($+0.07 \pm 0.01$) and HCO_3^- from baseline ($+3.9 \pm 0.9$ $mmol{\cdot}l^{-1}$), eliciting a state of metabolic alkalosis (7). Ergogenic effects have been reported in combat sports including boxing (8) and judo (9, 10) by either increasing punches landed or total work done (TWD). Whilst the effects of pre-exercise $NaHCO_3$ ingestion has been well-researched [for review see (11)], the effects of post-exercise ingestion between two bouts of exercise to promote recovery has received minimal attention. The use of this alternative method might permit a greater observed improvement in acid base balance during the recovery period, whilst the enhanced level of acid base balance would not have been utilized within the initial bout of exercise. These factors combined might therefore increase performance during the subsequent bout of exercise compared to pre-exercise $NaHCO_3$ ingestion. Indeed, Gough et al. (12) reported that 0.3 $g.kg^{-1}$ $NaHCO_3$ ingested 30 min into a 90 min post-exercise recovery period improved subsequent cycling time to volitional exhaustion by 33 s (∼14%) in recreationally active individuals. It is likely that an enhanced level of acid base balance was the primary mechanism for such an improvement, as the authors reported marked increases in pH and HCO_3^- prior to the second bout of exercise compared to the placebo [pH = +0.07, effect size (ES) = 2.6, HCO_3^- = +7 $mmol{\cdot}l^{-1}$, ES =3.4]. It is unknown, however, if these positive findings translate to other exercise modalities such as boxing, and individuals of a higher training status.

The mechanisms to explain the performance improvement following $NaHCO_3$ supplementation is not unique to changes in pH and HCO_3^-. Specifically, marked ionic shifts are suggested to contribute to muscle fatigue by impeding maximal Na^+, K^+-ATPase activity, subsequently impairing cell membrane excitability (2, 5, 13). Indeed, both large effluxes of extracellular K^+ concomitant with reductions in Na^+ have been suggested to exacerbate the K^+ induced decline in force production (14). Pre-exercise ingestion of $NaHCO_3$ has been shown to reduce K^+ and increase Na^+ prior to the onset of exercise (5, 8, 13, 15). Indeed, Siegler and Hirscher (8) reported $NaHCO_3$ supplementation prior to a simulated boxing protocol lowered K^+ compared to the placebo condition (4.0 ± 0.1 $mEq.l^{-1}$ vs. 5.3 ± 0.4 $mEq.l^{-1}$, respectively) and subsequently speculated that this reduction might have facilitated the resulting performance improvement. It is widely argued however, that electrolyte balance should be assessed by the collective analysis of the strong ion difference (SID), which is the balance of the fully dissociated cations and anions in intracellular and extracellular fluid (16). Synergistic changes in electrolytes are suggested to allow for deeper assessment of fatigue mechanisms, as opposed to reporting changes within a single electrolyte. In the only study to date, Gough et al. (17) reported a significant increase in the SID following $NaHCO_3$ supplementation and an improvement in 2 × 4 km time trial cycling bouts interspersed by 40 min recovery, although this study was conducted in a normobaric hypoxic environment. The purpose of this study therefore was to investigate the effects of post-exercise ingestion of $NaHCO_3$ on acid base balance recovery, the SID and subsequent boxing performance.

MATERIALS AND METHODS

Seven male elite professional boxers [age: 27.1 ± 5.1 years, stature: 175.8 ± 5.7 cm, body mass: 72.2 ± 10.3 kg, relative peak oxygen uptake (VO_{2peak}): 55.8 ± 11.4 $ml{\cdot}kg{\cdot}min^{-1}$] from various boxing weight classifications including flyweight, lightweight, junior welterweight (WBO/IBF) super lightweight (WBA/WBC), middleweight, and super middleweight completed this study. Participants were considered elite standard boxers and were at least Commonwealth (British Empire), English, International Masters, British Masters, or Midlands Area title holders, with an average of 4.1 ± 3.6 years professional boxing experience. At the time of data collection, all participants were in pre-competition training. The study received institutional ethics committee approval (University of Derby, UK) prior to any testing, and participants were informed of the details of the study, both verbally and in writing, prior to providing written informed consent in accordance with the Declaration of Helsinki. Physical Activity Readiness Questionnaire (PAR-Q) and blood analysis questionnaires were completed prior to each bout of exercise.

Preliminary Procedures

Prior to each trial, participants were requested to avoid strenuous exercise and to abstain from caffeine and alcohol ingestion for at least 24 h. Participants were also encouraged to adopt the same mixed balanced diet with adherence monitored through a food diary, which participants recorded 24 h prior to testing. A photocopy of the food diary was given to each participant to facilitate dietary replication prior to each experimental trial with 100% adherence achieved. Finally, participants were verbally screened to ensure they had refrained from ingestion of ergogenic buffers such as sodium citrate and β-alanine for 6 months prior to beginning the study.

Participant's body composition was assessed using Dual Energy X-ray Absorptiometry (Lunar iDXA, GE Healthcare, Hertfordshire, UK) 7–10 days prior to the experimental trials for analysis of body mass (kg). During the same visit, following 3 h of fasting, participants completed an incremental exercise test on a motorized treadmill (Desmo, Woodway, Germany) to assess peak oxygen uptake (VO_{2peak}). Initially participants warmed up for 5 min at 8 $km{\cdot}h^{-1}$ with a 0% gradient. The

TABLE 1 | Example of one round of the high intensity interval run (HIIR) protocol.

Exercise duration (sec)	~%VO_{2PEAK}	Intensity level
30	90	High
30	75	Moderate
30	90	High
30	75	Moderate
30	90	High
30	75	Moderate
30	90	High
30	75	Moderate
60 AR*	SS**	Low

AR, active recovery; SS**, self-selected during familiarization.*

TABLE 2 | Punch combinations sequence utilized during boxing specific performance.

Phase 1	Phase 2 (combinations)	Phase 3
MIR	J-	MOR
MIR	J-BH	MOR
MIR	J-BH-LU	MOR
MIR	J-BH-LU-BU	MOR
MIR	J-BH-LU-BU-LH	MOR
MIR	J-BH-LU-BU-LH- BHH	MOR

MIR, move in range; MOR, move out of range; J, Jab; BH, backhand; LU, lead uppercut; BU, backhand uppercut; LH, lead hook; BHH, backhand hook.

test began with a 3 min stage at 10 $km \cdot h^{-1}$, subsequently the speed increased by 2 $km \cdot h^{-1}$ every three min until it reached the 16 $km \cdot h^{-1}$ stage. From this point the gradient was increased by 2% every 2 min until volitional exhaustion. Throughout the test expired gas samples were collected via an online breath by breath system (Cortex MetaLyzer II, Biophysik, Leipzig, Germany) which was calibrated before each test as per the manufacturer's guidelines. Expired gas samples were analyzed for oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER). The highest value of VO_2 obtained in any 30 s period was used to calculate VO_{2peak}.

Familiarization

During the second laboratory visit participants were familiarized with the high intensity interval run (HIIR) protocol (**Table 1**), and the punch type techniques and combinations (**Table 2**), that would be utilized during experimental trials. In the HIIR, emphasis was placed upon exercising at a percentage of running velocity at VO_{2peak} during each differing work interval as opposed to heart rate ensuring the total time at each workload was readily matched. Finally, participants ran at a velocity that elicited 90% VO_{2peak} to volitional exhaustion (T_{LIM}) as a measure of high-intensity endurance capacity.

Experimental Design and Protocol

Experimental trials were conducted using a repeated measures, partially counterbalanced (due to odd number sample size), double-blind, and placebo controlled design, each separated by 7 days. Participants reported for each trial 3 h postprandial and at the same time of day to avoid any circadian rhythm effects on performance (18). Body mass was measured and recorded at the start of each laboratory visit (Seca 761 weight scales, Birmingham, UK), to monitor possible fluctuations between experimental trials due to the participants being in pre-competition training stages. The following baseline measures were obtained after 5 min seated rest: heart rate (HR); (Polar, FT40, Finland), blood lactate concentration [Bla^-], base excess (BE), bicarbonate ion concentration [HCO_3^-] and a range of electrolytes (sodium [Na^+], potassium [K^+], calcium [Ca^{2+}], and chloride [Cl^-]). The electrolyte data was used to calculate the apparent SID using an online spreadsheet (19) based on the following formula: $[K^+] + [Na^+] + [Ca^{2+}] + [Na^+] - [Cl^-] - [Bla^-]$. Blood variables were collected via a finger prick capillary blood sample and analyzed with a blood gas analyser (ABL90 Flex, Radiometer, West Sussex, UK). Perceived readiness to exercise (PRE) was then recorded against an 11 point (0–10) scale with 0 representing "not at all ready to exercise" and 10 representing "completely ready to exercise" (20).

Exercise trials commenced with a 5 min treadmill run at a velocity eliciting ~60% VO_{2PEAK} (warm-up) immediately followed by the HIIR protocol (**Table 1**) which was repeated three times to imitate the demands of 3 × 4 min boxing rounds, each separated by 60 s active recovery. A self-selected active recovery was recorded and replicated for each recovery interval in both experimental trials. Subsequently, a fourth and final bout was performed on a treadmill at a running velocity eliciting ~90% VO_{2PEAK} to volitional exhaustion (T_{LIM1}) with participants blinded from distance and time completed. Overall (i.e., related to cardiovascular strain) ratings of perceived exertion (RPE_O) (21) were recorded within the final 5 s of each round. Immediately post-exercise HR and RPE_O were recorded. Five min post-exercise HR and blood metabolite/electrolyte data was collected as previously described.

Participants then recovered passively for 75 min prior to undertaking subsequent boxing performance. This was selected due to previous data showing this time period is approximately when acid base balance returns to baseline following high-intensity exercise (12). Ten minutes into recovery, participants consumed either 0.3 $g.kg^{-1}$ body mass of $NaHCO_3$ or 0.1 $g.kg^{-1}$ body mass of sodium chloride (placebo; PLA) within a standardized 5 min period. This time period was selected due to the fear of vomiting if ingestion began immediately post-exercise, whilst a longer time period was not used as this may have allowed acid base balance to recover back to baseline values prior to ingestion (12). Both drinks were mixed in 4 $ml \cdot kg^{-1}$ body mass tap water and 1 $ml.kg^{-1}$ body mass of double strength no added sugar orange squash (Sainsbury's, London, UK) (20). Thirty minutes post exercise abdominal discomfort (AD) and gut fullness (GF) were recorded using an 11 point (0–10) scale, with 0 representing "empty" and "completely comfortable," and 10 representing "bloated" and "unbearable pain," respectively (20). Water was consumed *ad libitum* during recovery (mean 582 ± 40 ml).

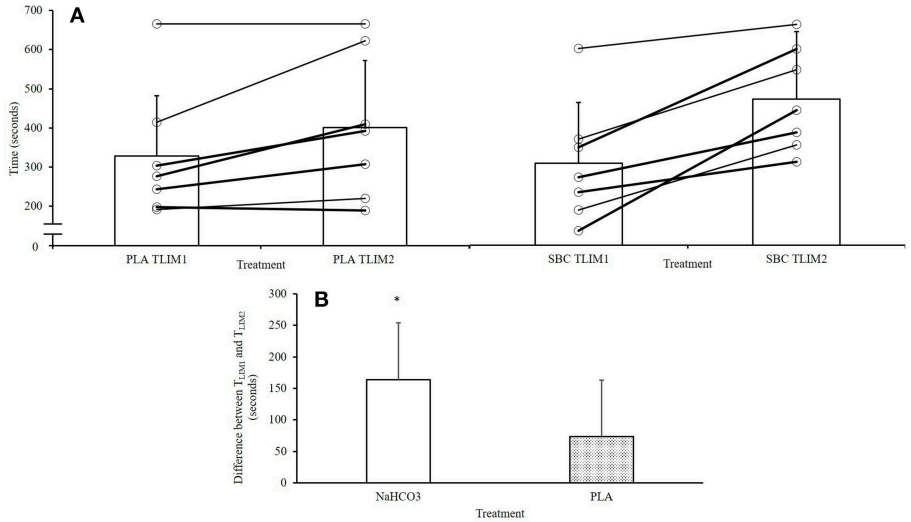

FIGURE 1 | Overview of performance responses following $NaHCO_3$ or PLA. *$NaHCO_3$ greater than PLA ($p < 0.05$). **(A)** Changes between T_{LIM1} and T_{LIM2}, **(B)** change in performance from T_{LIM1} and T_{LIM2} following $NaHCO_3$ or Placebo.

At the end of the 75 min recovery, HR, PRE, blood metabolites/electrolytes, AD, and GF were all recorded prior to participants performing a 5 min standardized dynamic warm up. Participants then completed the boxing specific protocol (**Table 2**) whereby they were required to strike the focus pads (Serious, Rapid Fire Punch Mitts, London, UK), which were worn by the same researcher for all trials. Each complete cycle consisted of 21 punches with participants instructed to stay in their preferred boxing stance (orthodox or southpaw) throughout. The punch combination cycle was performed repeatedly for 3 × 3 min rounds, each separated by 60 s passive recovery. Participants were all given the same boxing gloves (10 oz, Adidas, Hi-Tech Multi-Boxing Glove, Germany) for both experimental trials. An audio and visual boxing gym timer (Title Boxing, De luxe gym timer, USA) kept timing of rounds. Immediately at the end of each round participants HR, RPE_O and ratings of perceived exertion localized to the arms (RPE_A; Borg scale 6–20) were recorded. Upon completion of the 3 boxing specific rounds AD and GF were also recorded. Following a 60 s rest period, participants then performed a final high intensity treadmill run corresponding to a speed that elicited ∼90% VO_{2peak} to volitional exhaustion (T_{LIM2}). Immediately post exercise HR, RPE_O, AD, and GF were recorded, and 5 min post-exercise HR, blood metabolite/electrolytes, AD, and GF were recorded.

Statistical Analysis

Data was firstly checked for normality via a Shaprio-Wilk test, followed by a Mauchly test for homogeneity of variance/sphericity. A paired *t*-test was used for some performance (T_{LIM1} and T_{LIM2}) and blood/perceptual data (change in HCO_3^- during T_{LIM2}, change in HCO_3^- during recovery, and aggregated GI discomfort). A two-way [treatment × time] repeated measures ANOVA was conducted with a Bonferroni correction for changes in blood variables (pH, HCO_3^-, and lactate). Effect size (ES) for interactions from the ANOVA are reported as partial eta squared ($P\eta^2$), whilst between treatment ES are reported as Hedge's g effect sizes (*g*) [interpreted as per conventional thresholds described by (22)]. If $p < 0.05$ then 95% CI are reported, where changes that do not cross the zero boundary treated as significant. A Friedman test was used for non-normally distributed data (AD, GF), and where the a priori alpha value was observed (i.e., $p < 0.05$) a *post hoc* Wilcoxon signed rank-test was conducted with median, *z* score, and *p*-value reported. For non-normally distributed data the ES is calculated by $Z/\sqrt{n}$ with 0.10, 0.24, and 0.37 considered as small, medium, and large effects, respectively (23). Reproducibility of the performance in T_{LIM1} was assessed using intraclass correlation coefficients (ICC), with the *r*-value and significance reported. Additional statistics such as confidence intervals and effect sizes were used due to the small sample size in the study, which might not be suited to statistical procedures such as *t*-test and ANOVA in isolation. Data were analyzed using a statistical software package, SPSS (V.24, IBM Inc., Chicago, IL, USA).

RESULTS

Performance

Both initial bouts for T_{LIM1} were well-matched between PLA and $NaHCO_3$ (328 ± 155 vs. 307 ± 142 s; ICC: $r = 0.94$, $p = 0.002$; *t*-test, $p = 0.526$), showing that participants were at a similar level of fatigue at the start of the recovery period. Performance in T_{LIM2} was greater by 70 ± 90 s (28%) following $NaHCO_3$ compared to PLA ($p = 0.084$, CI = −153.8, 12.9; **Figure 1A**), with a moderate effect size ($g = 0.41$). The change in performance from T_{LIM1} to T_{LIM2} was greater following $NaHCO_3$ compared to PLA (+164 ± 90 vs. +73 ± 78 sec; $p = 0.02$, CI = 45.1, 428.8, $g = 1.0$; **Figure 1B**). One participant displayed an ergolytic effect

following $NaHCO_3$ ingestion, such that T_{LIM2} decreased by 13% compared to PLA (545 vs. 623 s). This participant also suffered from moderate to severe GI discomfort.

Blood Variables

No differences in pH between PLA and $NaHCO_3$ were observed at baseline (7.43 ± 0.04 vs. 7.42 ± 0.02; $p = 0.233$), or post T_{LIM1} (7.31 ± 0.04 vs. 7.31 ± 0.04; $p = 0.696$). Following the recovery period, and the ingestion of $NaHCO_3$, pH was greater prior to T_{LIM2} by 0.11 ± 0.02 units (1.4%) ($p < 0.001$, CI = 0.09, 0.13, $g = 3.4$). Post T_{LIM2}, no difference between treatments was observed for pH (7.31 ± 0.06 vs. 7.33 ± 0.08; $p = 0.271$; **Figure 2A**). There were no differences in HCO_3^- between PLA and $NaHCO_3$ at baseline (25.9 ± 1.5 vs. 26.0 ± 1.6 mmol.l^{-1}; $p = 0.750$), post T_{LIM1} (16.6 ± 2.2 vs. 16.8 ± 2.2 mmol.l^{-1}; $p = 0.723$), or post T_{LIM2} (17.7 ± 3.1 vs. 19.0 ± 3.4 mmol.l^{-1}; $p = 0.196$). Following recovery however, HCO_3^- was greater by 8.8 ± 1.5 mmol.l^{-1} (26.3%) post-$NaHCO_3$ supplementation compared to PLA ($p < 0.001$, CI = 7.3, 10.2, $g = 5.1$; **Figure 2B**). The change in HCO_3^- during recovery (post T_{LIM1} to pre T_{LIM2}) was greater following $NaHCO_3$ ingestion compared to PLA (16.6 ± 1.4 vs. 8.0 ± 2.1 mmol.l^{-1}; $p < 0.001$; CI = 6.5, 10.7, $g = 4.5$). During T_{LIM2}, the change in HCO_3^- during exercise was greater for $NaHCO_3$ compared to PLA (14.3 ± 2.9 vs. 6.9 ± 2.5 mmol.l^{-1} $p < 0.001$, 10.3, 4.5, $g = 2.5$). Post T_{LIM2}, BLa^- was 5.2 ± 2.6 mmol.l^{-1} (39.5%) greater following $NaHCO_3$ ($p = 0.002$, CI = 2.6, 7.3, $g = 2.0$), with no difference at any other time point ($p > 0.05$; **Figure 2C**).

Ingestion of $NaHCO_3$ caused marked changes in Na^+, K^+, Ca^{2+}, and Cl^- (**Figure 3**). A time*treatment interaction was observed for the SID ($p = 0.023$, $P\eta^2 = 0.576$), such that a 10% increase in the SID was observed post recovery following $NaHCO_3$ ingestion compared to PLA (46 ± 1 vs. 36 ± 4 meq/l; $p < 0.001$, CI = 6.3, 13.7, $g = 3.2$; **Figure 4**).

Heart Rate and Perceptual Measures

Post-exercise ingestion of $NaHCO_3$ increased HR in rounds 2 and 3 compared to PLA ($p < 0.05$), whilst no effect was observed on RPE_O or RPE_A ($p > 0.05$) during any round of the HIIR. No effect on post T_{LIM2} HR ($p = 0.217$, $g = 0.46$) was observed following $NaHCO_3$. Likewise, no difference in HR between $NaHCO_3$ and PLA were observed at any time point during T_{LIM2} (all $p > 0.05$). Similarly, $NaHCO_3$ supplementation had no effect on post T_{LIM2} RPE_O ($Z = 1.47$, $p = 0.383$), with no difference observed between treatments at any time point ($p > 0.05$).

Abdominal discomfort was greater following $NaHCO_3$ ingestion at 30 min recovery, displaying a moderate effect size (3.6 ± 3.0 vs. 1.6 ± 2.3; $Z = 1.76$, $p = 0.07$, $g = 0.7$). At the end of recovery, abdominal discomfort had generally reduced, although $NaHCO_3$ was still greater (1.7 ± 1.7 vs. 0.7 ± 1.3; $Z = -1.89$, $p = 0.06$, $g = 0.6$). No time*treatment interaction was observed for gut fullness ($p = 0.219$, $\eta^2 = 0.213$). Aggregated GI discomfort was not significantly different between $NaHCO_3$ ingestion and PLA (19 ± 13 vs. 13 ± 15; $p = 0.175$, −14.1, 3.2), although it was associated with a moderate effect size ($g = 0.40$; **Figure 5**).

DISCUSSION

This study investigated the effects of post-exercise $NaHCO_3$ ingestion on subsequent high-intensity boxing performance. Following $NaHCO_3$ ingestion, acid base balance was increased prior to T_{LIM2} compared to PLA which subsequently improved subsequent boxing specific exercise performance. Athletes and coaches can therefore implement this strategy to support training at times when multiple bouts of exercise are carried out with limited recovery interspersed.

The findings of the current study show that $NaHCO_3$ ingestion improved subsequent boxing specific performance, by markedly reducing the decline from T_{LIM1} to T_{LIM2}. This adds to previous work evaluating post-exercise $NaHCO_3$ supplementation as a recovery supplement (12). Indeed, Gough et al. (12) showed that $NaHCO_3$ ingestion 30 min into a 90 min recovery period improved subsequent cycling capacity, such that a moderate effect size ($g = 0.5$) was observed vs. the placebo within a group of recreationally trained males. The current study adds however, similar ergogenic effects can be achieved with post-exercise $NaHCO_3$ ingestion within a shorter recovery time, individuals of a higher training status, and combat exercise. In addition, these findings also support previous literature showing $NaHCO_3$ ingestion is an effective supplement to improve combat performance when ingested prior to exercise (8, 24). Future research could consider the impact of $NaHCO_3$ ingestion to enhance subsequent performance in other combat sports.

Based on the observed improvements it can be speculated that if $NaHCO_3$ supplementation could be adapted into a chronic weekly supplementation strategy, this might lead to greater adaptation to training. Previous work by Percival et al. (25) has shown mRNA expression of PGC-1a, a known mechanism for mitochondrial adaptation, was increased 3 h following a high intensity training session with acute $NaHCO_3$ ingestion compared to a placebo. Based on this evidence it is plausible that this may aid training adaptation in boxing, however, the study by Percival et al. (25) was in cycling and in lesser trained individuals to the current study (healthy men vs. elite boxers). In addition, other studies investigating the effects of $NaHCO_3$ ingestion to support training adaptations are equivocal within trained individuals. Indeed, Edge et al. (26) reported chronic $NaHCO_3$ ingestion significantly increased lactate threshold by 11% and time to fatigue (100% VO_{2peak}) by 41% compared to a placebo following 8 weeks of cycling interval training. Both Driller et al. (27) and Siegler et al. (28) however, have shown no greater training adaptations following $NaHCO_3$ ingestion within rowing and resistance exercise modalities across 4 and 10 weeks of training, respectively. Considering positive findings have been reported in combat exercise following $NaHCO_3$ ingestion (8, 24), further research could explore if greater training adaptations occur with chronic $NaHCO_3$ ingestion.

In the present study, the likely mechanism to explain the improvement in subsequent performance is the changes in blood acid base balance between bouts, such that pH, HCO_3^-, and the SID were significantly higher at 75 min recovery following $NaHCO_3$ ingestion. Full recovery of pH, HCO_3^-, and the SID was achieved in approximately 30–35 min. This is in contrast

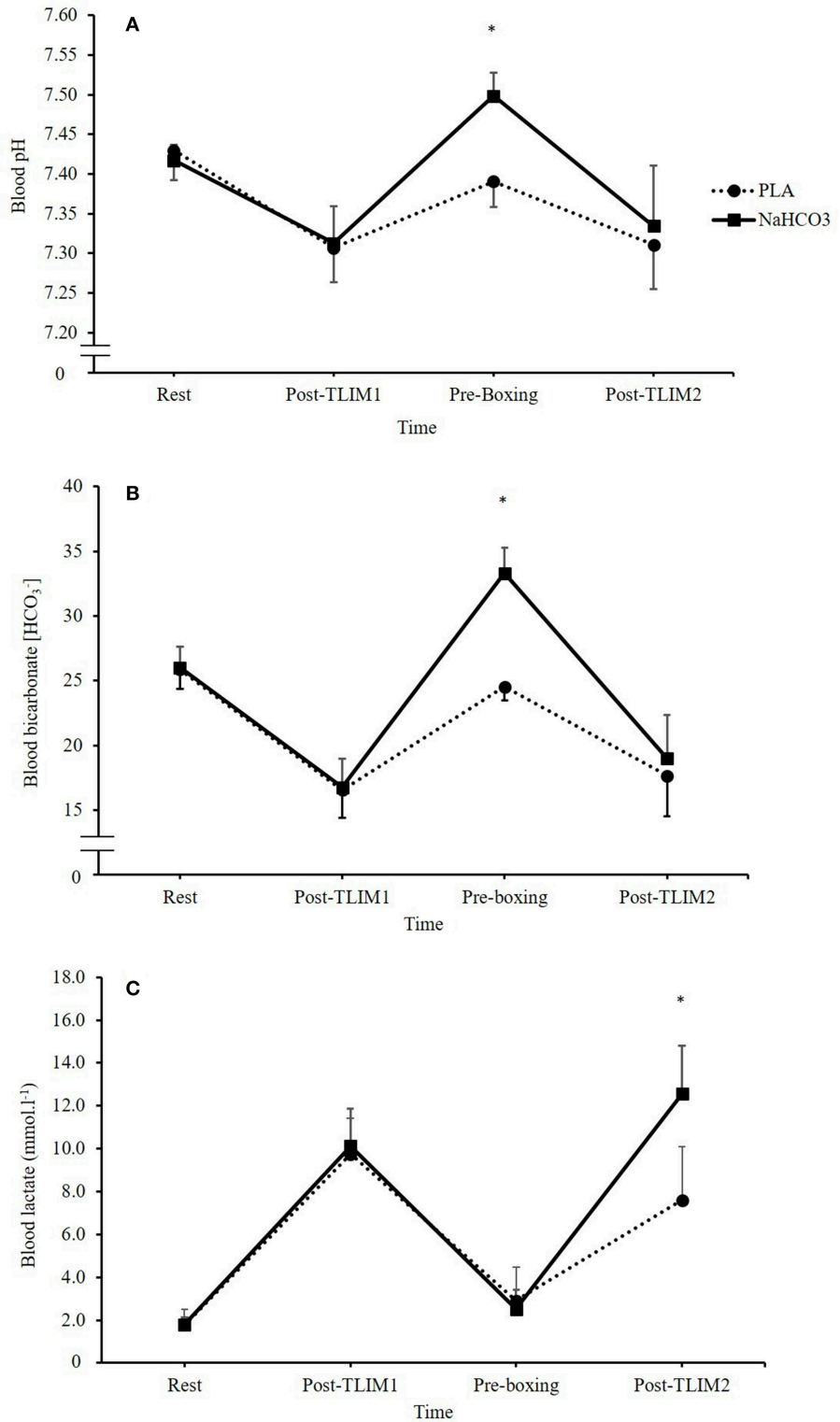

FIGURE 2 | Blood acid base balance responses following $NaHCO_3$ or PLA, where **(A)** pH, **(B)** blood bicarbonate [HCO_3^-], and **(C)** blood lactate [BLa^-]. *$NaHCO_3$ greater than PLA ($p < 0.05$).

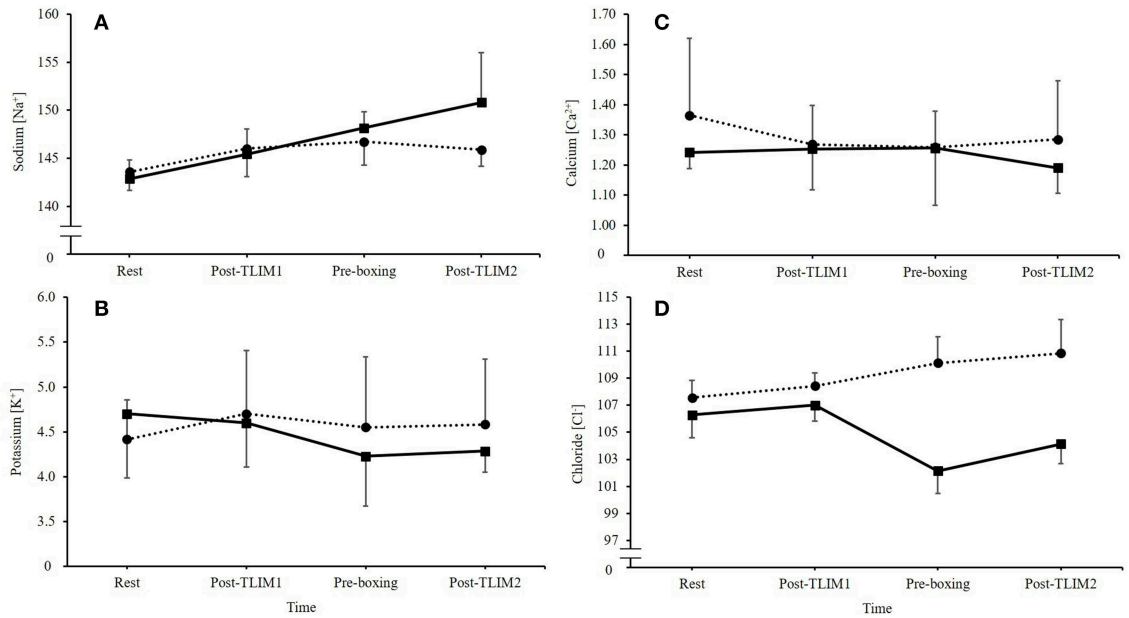

FIGURE 3 | Changes in extracellular electrolytes following $NaHCO_3$ or PLA, where **(A)** sodium [Na^+], **(B)** potassium [K^+], **(C)** calcium [Ca^{2+}], and **(D)** chloride [Cl^-].

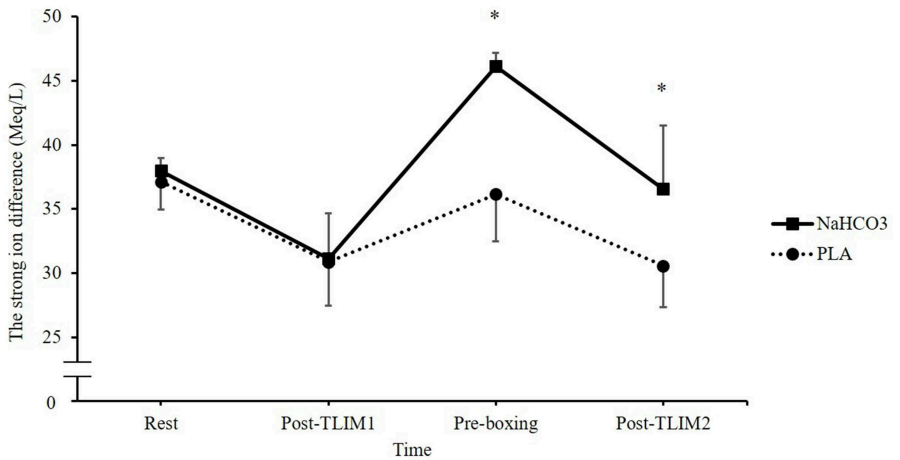

FIGURE 4 | Changes in blood strong ion difference (SID) following $NaHCO_3$ or PLA. *$NaHCO_3$ greater than PLA ($p < 0.05$).

to the placebo condition, which failed to recover any of these blood analytes to baseline within 75 min of recovery. As a result, $NaHCO_3$ ingestion mitigated the disturbance to acid base balance during T_{LIM2}, which subsequently may explain the performance improvement. Such a greater state of metabolic alkalosis has been shown to increase buffering capacity by facilitating efflux of H^+ from the active muscle by enhanced circulating HCO_3^-, and thus, increasing the glycolytic energy contribution to high-intensity exercise (24, 29). The current study supports these mechanisms, reporting a 2-fold increase in the HCO_3^- change during T_{LIM2}, and a marked increase in lactate post-T_{LIM2} following $NaHCO_3$ ingestion. Indeed, Lopes-Silva et al. (24) showed similar changes in post-exercise lactate following $NaHCO_3$ ingestion, but also reported a significant 31% increase in estimated glycolytic activity during simulated taekwondo combat. It is important to note however, the link between metabolic acidosis and fatigue has been widely criticized, suggesting at physiologically valid muscle temperatures, accumulation of H^+ has limited effects on muscle contractile ability (30). As the current study did not assess either temperature or metabolite accumulation in muscle, we cannot confirm that acidosis has a direct impact on fatigue and performance.

An alternative mechanism to explain the performance improvement might be the increases in the SID following

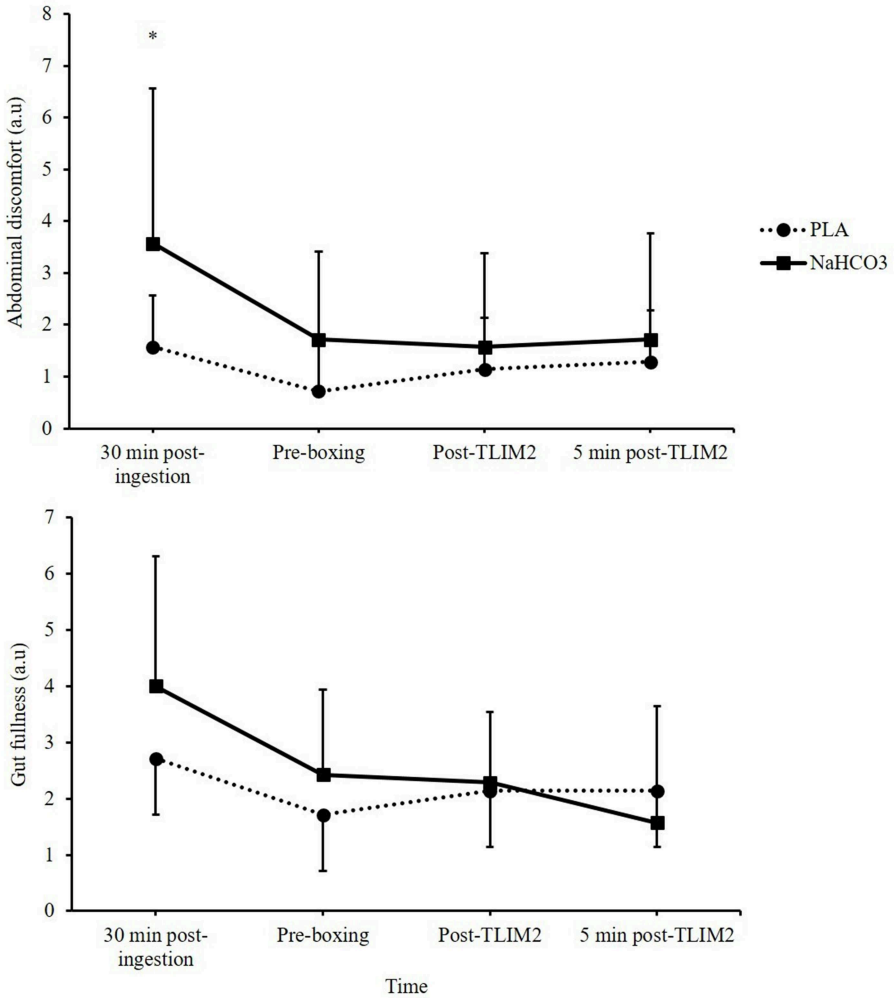

FIGURE 5 | Gastrointestinal (GI) discomfort (gut fullness and abdominal discomfort) following $NaHCO_3$ or PLA. *$NaHCO_3$ greater than PLA ($p < 0.05$).

$NaHCO_3$ ingestion. Reductions in K^+ and Cl^- were observed, whilst Na^+ was increased in the recovery period, which lead to an overall increase in the SID. This could lead to an increase in electrical excitation, membrane potentials and muscle action potentials, which in turn, could support maximal Na^+, K^+-ATPase activity (2, 31). Previous research, however, has suggested the most important electrolyte change is K^+, by demonstrating that raised extracellular concentration depresses muscle excitability (31). This suggests that the important changes that $NaHCO_3$ supplementation elicits is in K^+. Nonetheless, shifts in Cl^- similar to those observed in the present study have been suggested to drive K^+ back to the muscle fiber through inward rectifier channels, which assist in returning the cell back to resting membrane potential (32). A well-designed study by Bouclin et al. (14) also showed that when an increased K^+ and reduced Na^+ were altered in combination, the effects on twitch and tetanic contractions were greater than the changes in these ions in isolation. It is more likely therefore, that collective changes in electrolyte regulation explain the ergogenic mechanism of $NaHCO_3$ supplementation. Further research should therefore continue to explore the effects of $NaHCO_3$ supplementation on the SID and exercise performance.

One individual presented moderate to high GI discomfort following $NaHCO_3$ ingestion and displayed an ergolytic effect on performance. These findings agree with prior investigations suggesting GI discomfort might be a factor that negates the performance improvement from $NaHCO_3$ (33–35). Indeed, Saunders et al. (33) reported upon removing participants who suffered GI discomfort following $NaHCO_3$ ingestion, only then did total work done (TWD) improve ($p = 0.01$, $d = 0.25$) compared to when all participants were included ($p = 0.16$, $d = 0.14$). However, performance benefits in combination with the onset of GI discomfort have occurred previously, whilst there is a lack of a direct link between GI discomfort and exercise performance following $NaHCO_3$ ingestion (20, 36). Individuals that suffer from severe GI discomfort could benefit from a

lower dose of $NaHCO_3$, as 0.2 $g.kg^{-1}$ BM $NaHCO_3$ has been shown to produce similar ergogenic responses whilst significantly reducing GI discomfort (36). Alternatively, the athlete could consider gastric bypass methods of delivery (i.e., enteric coated capsules), as novel data has suggested this may be suitable to reduce GI discomfort but still achieve the required increase in acid base balance (37, 38); although the performance responses are currently unclear. Further research should explore both lower doses of $NaHCO_3$ and the use of gastric bypass methods of delivery to understand the link between GI discomfort and performance following $NaHCO_3$ ingestion.

A limitation of this study is the small sample size, meaning further work is required to establish the impact of manipulating post-exercise acid base balance on performance and recovery. Despite this, the participant cohort were of an elite standard which are typically difficult to access. The current study findings therefore still have high practical application in sports performance, although further research with larger sample sizes are required. These findings compliment previous research investigating $NaHCO_3$ supplementation and exercise performance within lesser-trained combat athletes (9, 24, 39) and support the use of $NaHCO_3$ supplementation to promote superior recovery.

CONCLUSION

The use of $NaHCO_3$ is a suitable ergogenic aid to achieve a greater magnitude of acid base balance recovery and improve subsequent boxing performance within elite level boxers. Being the first study to assess this within an elite participant cohort, the results of this study are of significance to athletes and coaches in an applied setting. Boxers within the elite category could therefore implement this strategy to augment training performance and potentially the subsequent adaptations. One participant did present ergolytic effects following $NaHCO_3$ ingestion however, which seemed to be due to high GI discomfort. Athletes should therefore trial $NaHCO_3$ ingestion to assess individual tolerability. Future research should implement similar recovery interventions within a larger sample of elite athletes to explore the effectiveness of $NaHCO_3$ supplementation as a recovery strategy.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The study received institutional ethics committee approval (University of Derby, UK) prior to any testing, and participants were informed of the details of the study, both verbally and in writing, prior to providing written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

This study was conceived by MH and designed by MH and SR. Data were collected by SR and analyzed by LG and MH. Data interpretation and manuscript preparation were undertaken by LG, MH, LM, and SS. All authors approved the final version of the paper.

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ORIGINAL RESEARCH
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Short-Duration Beta-Alanine Supplementation Did Not Prevent the Detrimental Effects of an Intense Preparatory Period on Exercise Capacity in Top-Level Female Footballers

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Purpose: High-intensity activity is an important aspect of football performance during competitive match play. The aim of this study was to investigate the effect of beta-alanine supplementation throughout a short-duration intense football-specific training period prior to an international competition on measures of high-intensity running performance.

Methods: Twenty-four elite international U20 female footballers (age 18 ± 1 y, height 1.67 ± 0.07 m, body mass 62.7 ± 7.4 kg) volunteered to perform the YoYo Intermittent Recovery Test Level 1 (YoYo IR1), the Running Anaerobic Sprint Test (RAST) and a 20-m maximal sprint test on two separate occasions, separated by 3 weeks of training and supplementation. Participants were randomly assigned to receive either 6.4 g·day^{-1} sustained-release beta-alanine (BA, $N = 12$) or an equivalent dose of maltodextrin (placebo, PL, $N = 12$) throughout a 3-week standardized training camp.

Results: There was a main effect of group ($P = 0.05$) and time ($P = 0.004$) on YoYo IR1; overall values were lower in PL and distance covered was lower post- vs. pre-supplementation. There was no group × time interaction ($P = 0.07$). There was an effect of sprint number for RAST, but no further main effects and there were no effect for the 20-m sprint.

Conclusions: Top-level female footballers involved in this intense 3-week training period prior to a competition worsened their high-intensity intermittent exercise capacity, and this negative result was not attenuated by a short-duration BA supplementation protocol throughout the same period. Further work is necessary to elucidate whether adapted training protocols and BA dosing regimens could lead to better results.

Keywords: football training, nutritional supplementation, YoYo intermittent recovery test, repeated sprints, competition, fatigue, elite

INTRODUCTION

Football, also commonly termed as soccer, is the world's most popular sport, practiced by men and women all around the world (1). The women's game has seen a stark increase in both popularity and professionalism over the past decade, with research into the physiological demands of the women's game following a similar rise in popularity (2). The general characteristics of women's football demonstrate that match-play is predominantly performed at low-intensity activities interspersed by numerous high-intensity actions throughout (2, 3). Key moments that can affect the outcome of a game generally occur at high-intensities. High-intensity efforts are reduced during various phases of international matches and vary according to position (4) and top-level women players have been shown to perform more high-intensity running and sprints during games than their less successful counterparts (5). High-intensity activity, therefore, appears to be an important aspect of football performance during competitive match play.

Several field tests are employed to evaluate the training status of football players and are commonly used to predict match performance and determine the effectiveness of a training intervention. The YoYo Intermittent Recovery Tests (Level 1 [YoYo IR1] and 2 [YoYo IR2]) evaluate an individual's capacity to repeatedly perform and recover from intense exercise bouts, and is applicable to team sports players due to the specificity of the exercise undertaken (6). The Yo-Yo IR1 and IR2 have been shown to correlate to various variables of match performance and can be used as an indicator of the physical performance of elite female players throughout competitive matches (7, 8), making them appropriate models to examine the effect of any intervention designed to manipulate changes in performance during team sports. In addition to differentiating between playing standard, these tests can be used to monitor training adaptations, seasonal variation and determine differences between playing position [for review, see (9)]. The running anaerobic sprint test (RAST) is another protocol that has been shown to be reliable and valid to assess anaerobic power and is a good predictor of short-distance running performances (10) while the 20-m sprint test is a commonly used measure to assess team sport players (11). Any changes in these performance measures may be reflective of an enhanced capacity to improve in-match performance and thus are useful tools to determine the efficacy of any intervention.

The preparation period prior to an international competition is a delicate one in which fitness training must ensure a maintenance and rebuilding process following an intense season to ensure peak condition for the subsequent intense period of matches (12). During this phase there may also be an additional focus on technical and tactical preparation as opposed to intense physical conditioning (13).This is commonly referred to as the taper, which involves reducing the training loads from a previously intense program to optimise recovery and maximise performance (14, 15). Athletes commonly employ supplementation methods to enhance any adaptations from training routines. Beta-alanine is an amino acid that is ingested over several weeks to increase muscle carnosine content (16) and improve exercise capacity and performance (17). It is considered an effective ergogenic aid by the International Olympic Committee (18), although the effects of beta-alanine on football-specific protocols is unclear and contradictory. Beta-alanine has previously improved YoYo IR2 performance in amateur male footballers throughout a competitive season (19), although YoYo IR1 was not improved in young elite male basketball players (20). Evidence to support beta-alanine supplementation during shorter-duration repeated sprints is distinctly lacking (21–23) although supplementation alongside plyometric training did lead to greater improvements in RAST than training alone in female soccer players (11). This suggests that the combination of training and beta-alanine may be additive, something previously demonstrated with cycling sprint training (24), although no study to date has investigated the combined effect of a football-specific training period alongside beta-alanine supplementation on football-specific performance in females.

The aim of this study was to investigate the effect of beta-alanine supplementation throughout a short-duration intense football-specific training period prior to an international competition on measures of high-intensity running performance. We hypothesised that supplementation would lead to greater improvements in exercise measures than any seen with training alone.

METHODS

Participants

Twenty-four elite international under-20 (U20) female footballers (age 18 $\pm$ 1 y, height 1.67 $\pm$ 0.07 m, body mass 62.7 $\pm$ 7.4 kg) from different clubs competing in the elite divisions of the Brazilian football pyramid that were part of the national Brazilian team preparing for the South American U20 Women's Championship, volunteered for the study and were randomly assigned to receive either beta-alanine (BA, $N = 12$) or placebo (PL, $N = 12$). Subjects had not taken any creatine supplement in the 3 months prior to the study and had not taken BA for at least 6 months. None of the subjects were vegetarian and, therefore, would have encountered small amounts of beta-alanine in their diet from the hydrolysis of carnosine and its methyl derivatives in meat. The study was approved by the institution's Ethical Advisory Committee.

Experimental Design

All athletes in this study routinely performed the exercise protocols as part of standard fitness testing throughout their respective seasons. Participants performed the YoYo IR1, RAST, and 20-m sprint test on two separate occasions, separated by 3 weeks of training and supplementation. The exercise tests were performed in a standardized order: Sprint Test and the YoYo IR1 in the morning and the RAST test in the evening. All players performed the same 3-week standardized training program, which consisted of 1 to 2 training sessions per day and received 5 meals per day at standardized timepoints. Sleeping and waking times during the training period were controlled and identical for all athletes. Training and diet, including caffeine consumption, in the 24 h period prior to the first main exercise

session were standardized and the athletes repeated this prior to the second main session.

Supplement group allocation was conducted in blocks with groups being equalized according to performance in the YoYo IR1. Throughout the same 3-week period, participants were supplemented with either 6.4 g·day^{-1} of beta-alanine (CarnoSyn^{TM}, NAI, USA) or placebo (maltodextrin; NAI, USA) in sustained-release tablets. The dosing regimen consisted of two 800 mg BA or PL tablets ingested four times per day (7 AM/12 PM/5 PM/10 PM). Participants ingested the supplements alongside their standardized meals and a final dose before bed, ensuring all had 100% compliance to the supplementation regimen. No participant in either group reported any symptoms of paraesthesia throughout supplementation.

Experimental Procedures

YoYo Intermittent Recovery Test – Level 1

The YoYo IR1 consists of repeated 2 × 20 m runs between markers at progressively increasing speeds dictated by an audio signal. At the end of each 2 × 20 m bout, individuals performed 10 s of active recovery between consisting of a 10 m (2 × 5 m) walk. The test ended if the player failed to reach the finish line within the given time frame on two consecutive occasions or if the player felt unable to continue (volitional exhaustion). The total distance covered (m) during the test was recorded as the outcome measure.

Running Anaerobic Sprint Test (RAST) and 20-m sprint

The RAST consisted of seven 20-m maximal sprints with a passive 10 s recovery period between each sprint; the start of each sprint was indicated by a beep from the photocell equipment (CEFISE standard photocells, Brazil) which measured run time for every individual sprint. The photocells were connected to a computer with specific software (CEFISE, Brasil) for speed analysis. Outcome measures were sprint time of each sprint (s), total sprint time (s), mean, maximum and minimum power output (W) [calculated as Power = (Body Mass × Distance^2)/Time^3] and fatigue index (FI, %) [calculated as FI = (peak power - minimum power/peak power) × 100)] (10). Participants also performed 3 separate attempts of a maximum 20 m sprint test, with 5 min passive recovery between efforts. To start the sprint, the volunteer was positioned 1 m behind the first photocell to prevent premature activation of the timer. The timing of the start of each maximal sprint was determined by the athlete.

Data Analysis

Data were analysed using the SAS statistical package (SAS® University Edition, SAS Institute Inc., USA), and are presented as mean ± 1SD unless stated. Exercise data were analysed using mixed model analysis with individuals assumed as a random factor and supplementation (2 levels; BA and PL) and time (2 levels; Day 0 and 20) assumed as fixed factors. Repeated sprints during the RAST were analysed using mixed model analysis with individuals assumed as a random factor and supplementation (2 levels; BA and PL), time (2 levels; Day 0 and 20) and sprint number (7 levels; 0-7) assumed as fixed factors. Tukey–Kramer adjustments were performed when a significant F value was obtained, and the significance level was set *a priori* at $P \leq 0.05$. Individual responses for the YoYo IR1 were calculated according to time-to-completion using the spreadsheet of Swinton et al. (25) using 90% confidence intervals, a typical error calculated from reproducibility data (26) and a smallest worthwhile change of 0.2 × the standard deviation of the control session (27). Due to issues unrelated to the intervention (due to minor illness or injury, the coaches instructed the athletes not to complete all protocols as a precautionary measure), complete data for the YoYo IR1 was obtained for 20 athletes (BA = 10, PL = 10) and 22 athletes completed the 20-m sprint (BA = 11, PL = 11); all athletes completed the RAST pre- and post-supplementation.

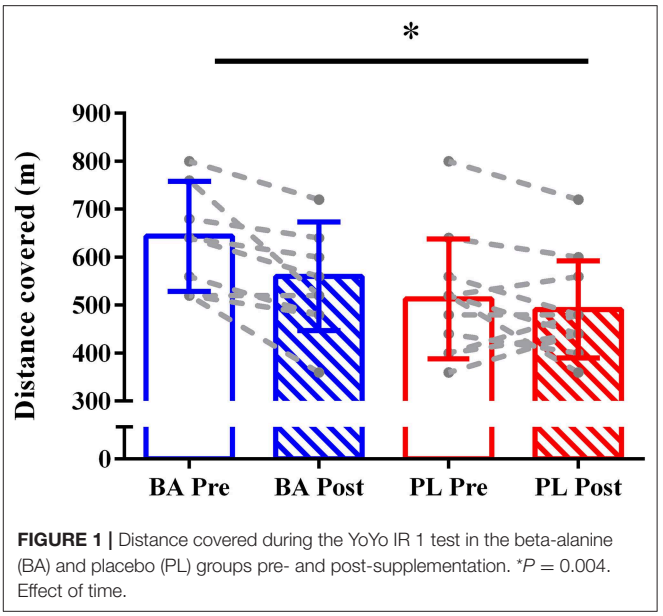

FIGURE 1 | Distance covered during the YoYo IR 1 test in the beta-alanine (BA) and placebo (PL) groups pre- and post-supplementation. *$P = 0.004$. Effect of time.

RESULTS

YoYo IR1

YoYo performance was not significantly different between groups at baseline (BA: 644 ± 114 m, PL: 513 ± 125 m; $P = 0.07$), although this almost reached statistical significance. This might be due to missing data (2 individuals from BA and 2 from PL). There was a main effect of group ($P = 0.046$), with lower overall values in the PL vs. BA group, and time ($P = 0.004$); distance covered was lower post- *versus* per-supplementation (−7.4 ± 14.4%). The group × time interaction did not reach statistical significance ($P = 0.07$; **Figure 1**). Individual data analysis revealed that no individuals in either group improved performance above the smallest worthwhile change during the YoYo IR1, although two athletes in BA and one in PL worsened performance.

RAST and 20 m Sprint

There was no effect of group ($P = 0.67$) or time ($P = 0.45$) for sprint times during the RAST, but there was an effect of sprint number ($P < 0.0001$), reflecting an increase in time to complete each sprint with increasing sprint number (**Table 1**). There were

TABLE 1 | Sprint times (s) during the RAST for the beta-alanine (BA) and placebo (PL) groups pre- and post-supplementation.

	BA		PL	
	Pre	Post	Pre	Post
Sprint 1 (s)	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.6 ± 0.1
Sprint 2 (s)	3.6 ± 0.2	3.6 ± 0.1	3.6 ± 0.1	3.6 ± 0.1
Sprint 3 (s)	3.7 ± 0.1*	3.7 ± 0.2*	3.6 ± 0.1*	3.7 ± 0.2*
Sprint 4 (s)	3.8 ± 0.2*	3.8 ± 0.2*	3.7 ± 0.1*	3.7 ± 0.2*
Sprint 5 (s)	3.8 ± 0.1*	3.8 ± 0.2*	3.8 ± 0.1*	3.8 ± 0.2*
Sprint 6 (s)	3.9 ± 0.2*	3.9 ± 0.2*	3.8 ± 0.1*	3.9 ± 0.2*
Sprint 7 (s)	3.9 ± 0.1*	3.9 ± 0.2*	3.9 ± 0.1*	3.9 ± 0.1*
Total sprint time (s)	26.1 ± 1.0	26.2 ± 1.0	26.0 ± 0.6	26.0 ± 1.0
20-m sprint	3.5 ± 0.1	3.5 ± 0.2	3.5 ± 0.1	3.4 ± 0.1

*$P < 0.0001$ Effect of sprint, *indicates a significant difference from Sprint 1.*

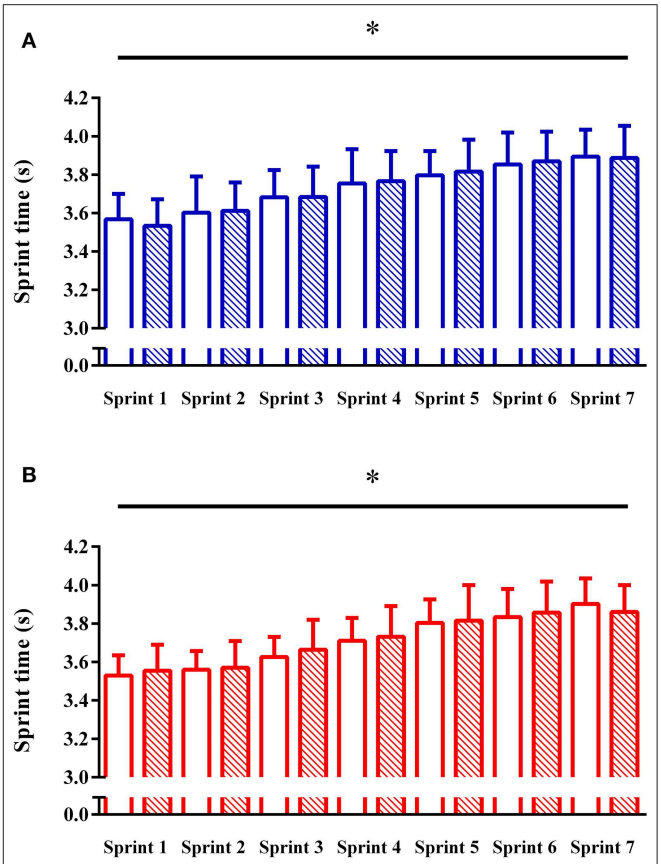

FIGURE 2 | Sprint performance during the RAST for the beta-alanine **(A)** and placebo **(B)** groups pre- (clear bars) and post- (striped bars) supplementation. *$P < 0.0001$. Effect of sprint number.

no group × time × sprint interaction effects for sprint times ($P = 0.96$). There were no group, time or group × time interactions for total time, maximum, mean and minimum power and fatigue index during the RAST (all $P > 0.05$) (**Figure 2**). There was no effect of group ($P = 0.91$), time ($P = 0.50$) or group × time interaction ($P = 0.25$) for the 20-m sprint test.

DISCUSSION

This study aimed to investigate the effect of BA supplementation in high-level Brazilian female soccer players during a three-week preparatory training period. The main findings showed that responses to BA supplementation were not different from those obtained with placebo and were unable to avoid decreases in performance during the YoYo IR1, which likely occurred due to high workloads imposed in this preparatory period. There were no changes in repeated or maximal sprint performance.

The training load employed with these athletes resulted in a reduced exercise capacity during the YoYo IR1 (−7.4%). Prior to major events, such as an international tournament, it is common to taper, namely reducing the training load from a previously intense program in order to optimise gains and recovery and maximise subsequent performance (14, 15). Our data suggest that, not only was the training intervention too intense in nature to illicit improvements in exercise capacity, it actually worsened performance which is contrary to the aims of the training. Previous data from an under-20 female football team preparing for the World Cup showed a progressive improvement in YoYo IR1 performance leading up to competition (Tunstall H, personal communication in (6)), although the authors suggest this was reflective of a more focused fitness training schedule and the low starting fitness levels of these female players. In the current study, our athletes all plied their trade for top-level national sides, and most were regular starters for their respective teams. It is possible that a long grueling season took its toll on the players, and that performance was a result of accumulated fatigue over the season and would have reduced over this three-week period regardless of the intense training. In fact, YoYo IR1 performance of these athletes prior to the training and supplementation intervention was lower than that shown previously in elite female footballers (7, 9), which provides support for this theory.

Short-term BA supplementation (3 weeks) was unable to attenuate this training-associated decline in YoYo IR1 performance. The lack of an effect shown here is line with previous research showing no changes in YoYo IR1 in young elite male basketball players with BA supplementation (20), although BA did improve YoYo IR2 (19) in amateur male footballers. The YoYo IR2 is initiated at a higher intensity than the YoYo IR1, with a higher contribution from anaerobic glycolytic pathways increasing the contribution of buffering capacity to performance (9), making it more susceptible to improvements with BA. It is also possible that the relatively short loading period in this study did not meet the threshold necessary for a sufficient increase in muscle carnosine to elicit performance improvements. Smith et al. (28) showed similar improvement in cycling capacity following 3 weeks of high-intensity interval training with both BA and PL, but greater improvements from weeks 3–6 were shown with BA. It is currently unknown what the minimal necessary increase in muscle carnosine is to elicit a performance improvement (29) and any definitive conclusions here are not

possible due to the lack of muscle carnosine content analysis in the current study. Had the athletes commenced supplementation prior to the training phase, thus ensuring increased muscle carnosine content prior to the intense training period, it is possible that results may have been different. However, we were unable to enforce the supplementation protocol prior to the international period during which we had access to the players, a potential consequence of working with elite club players on international duty. As it stands, short-term BA supplementation was unable to attenuate the decline in YoYo IR1 performance following the training period in this study.

Neither training nor supplementation led to changes in repeated sprint ability or sprint performance. These data are in line with previous studies showing no effect of BA on short-duration repeated sprints in team sports players (21, 22). However, previous research in female football players has shown BA supplementation to improve mean power output during repeated 30-s Wingate sprints (30) and induce greater improvements in repeated-sprint tests when combined with plyometric training compared to training alone (11). It is possible that the highly trained nature of our athletes contributed to these results, since meta-analytical data has shown well-trained individuals to achieve smaller performance gains with supplementation than non-trained individuals (17). The aforementioned studies recruited university level (30) and amateur (11) players, while we employed elite youth players. It is also important to again emphasise that the lack of any changes in these tests may similarly be due to the intense nature of the training program, inhibiting any potential adaptations with or without supplementation.

One of the strengths of this study is that it was a real-world intervention in which we implemented a double-blind placebo-controlled supplementation protocol in top-level female athletes who were part of a competitive international set-up performing their normal pre-competition training program. The standardized pre-tournament training camp provided a unique environment that required all athletes to undergo identical daily routines such as training, nutritional intake and sleep, thus removing several variables which could contribute to individual variability. Indeed, our data showed striking consistency with all but one individual in BA showing a reduction in distance covered during the YoYo IR1, while six athletes in PL also covered less distance; however, statistical analysis revealed that only two in BA and one in PL could be considered to have worsened performance with >90% certainty (25). The controlled nature of this study has great practical applicability to similar athletes undergoing these types of intervention by showing BA to be ineffective during such a short and intense training period. However, alternative methods might be implemented, such as lighter training loads and earlier implementation of the supplementation regime, which might lead to different results. Indeed, this study also highlights the delicate nature of working with an international team since we could not make any changes to their usual routines until their individual seasons with their respective national clubs had ended.

PRACTICAL APPLICATIONS

Intense preparatory training periods prior to international competitions may place unnecessary strain on top-level footballers following a grueling season. Our data suggest that international teams may inadvertently overload their players leading to a reduced high-intensity exercise capacity in these players. Reduced exercise capacity was not counteracted by short-duration BA supplementation, although it is possible that the supplementation protocol was sub-optimal. Perhaps more communication between clubs and international teams may facilitate this process and avoid overreaching or overtraining, while prior initiation of the supplementation protocol would further benefit any potential adaptation.

CONCLUSIONS

Top-level female footballers involved in this intense 3-week training period prior to a competition worsened their high-intensity intermittent exercise capacity, and this negative result was not attenuated by a short-duration BA supplementation protocol throughout the same period. Further work is necessary to elucidate whether adapted training protocols and BA dosing regimens could lead to better results.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of São Paulo. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GR, AR, EP, VC, MB, PG, BG, and BS contributed to the conception and design of the study. RR, EP, and BS organized the database and performed the statistical analysis. RR, BD, AG, EP, and BS wrote the first draft of the manuscript. GR, AR, VC, MB, PG, and BG contributed to the subsequent versions. All authors contributed to manuscript revision, and read and approved the final submitted version.

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Conflict of Interest: BS has previously received financial support from Natural Alternatives International (NAI), a company that produces BA, to undertake a study unrelated to this one. NAI has also provided BA supplements free of charge for this and further experimental investigations and supported open access page charges for numerous publications involving the authors. NAI have not had any input (financial, intellectual, or otherwise) into this original investigation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

SYSTEMATIC REVIEW
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The Muscle Carnosine Response to Beta-Alanine Supplementation: A Systematic Review With Bayesian Individual and Aggregate Data E-Max Model and Meta-Analysis

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Beta-alanine (BA) supplementation increases muscle carnosine content (MCarn), and has many proven, and purported, ergogenic, and therapeutic benefits. Currently, many questions on the nature of the MCarn response to supplementation are open, and the response to these has considerable potential to enhance the efficacy and application of this supplementation strategy. To address these questions, we conducted a systematic review with Bayesian-based meta-analysis of all published aggregate data using a dose response (Emax) model. Meta-regression was used to consider the influence of potential moderators (including dose, sex, age, baseline MCarn, and analysis method used) on the primary outcome. The protocol was designed according to PRISMA guidelines and a three-step screening strategy was undertaken to identify studies that measured the MCarn response to BA supplementation. Additionally, we conducted an original analysis of all available individual data on the MCarn response to BA supplementation from studies conducted within our lab (n = 99). The Emax model indicated that human skeletal muscle has large capacity for non-linear MCarn accumulation, and that commonly used BA supplementation protocols may not come close to saturating muscle carnosine content. Neither baseline values, nor sex, appeared to influence subsequent response to supplementation. Analysis of individual data indicated that MCarn is relatively stable in the absence of intervention, and effectually all participants respond to BA supplementation (99.3% response [95%CrI: 96.2–100]).

Keywords: nutrition, physiology, metabolism, supplement, meta-analysis, histidine containing dipeptides, buffering, dosing

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INTRODUCTION

Beta-alanine (BA) supplementation is a widely used dietary strategy, due to its proven efficacy in increasing skeletal muscle carnosine content (MCarn) (Harris et al., 2006). Carnosine is a dipeptide formed from the amino acids beta-alanine (BA) and L-histidine, and is present in high concentrations in human skeletal muscle (∼20–30 mmol·kg^{-1} dry muscle). Its purported roles include: proton buffering (Dolan et al., 2018), anti-oxidation (Boldyrev et al., 2010), anti-glycation (Ghodsi and Kheirouri, 2018), metal chelation (Boldyrev et al., 2013), and influencing calcium sensitivity (Dutka and Lamb, 2004), and hence muscle contractility. These diverse physiological properties allow carnosine to contribute to multiple processes in skeletal muscle metabolism, and considerable research efforts have been made to investigate both means to increase it, and in what situations such increases are beneficial. BA availability is the limiting factor in intramuscular carnosine synthesis (Harris et al., 2006) and it is widely recognized that supplementation with this amino acid substantially increases MCarn. This supplementation strategy has proven efficacious in many situations, with the majority of research focusing on its ergogenic properties (Hill et al., 2007). A strong body of literature attests to the ability of BA supplementation to improve high-intensity exercise performance, with meta-analytic data indicating that it exerts its greatest ergogenic influence in capacity-based exercise tests that last between 30 s and 10 min (Saunders et al., 2017a). This ergogenic effect likely occurs due to MCarn's buffering action (Boldyrev et al., 2013; Dolan et al., 2019a), and has earned BA its place as one of the world's most popular, scientifically-backed and widely endorsed sports supplements available (Maughan et al., 2018). The therapeutic efficacy of BA supplementation is less well-investigated, although it appears to be a promising strategy, given its ability to increase MCarn, which in itself, may, potentially, have numerous therapeutic applications (Artioli et al., 2018), including roles in anti-senescence (Boldyrev et al., 2010), neuroprotection (De Marchis et al., 2000; Dobrota et al., 2005), tumor growth attenuation (Renner et al., 2010), improved clinical outcomes in participants with Parkinson's disease (Boldyrev et al., 2008) and enhanced glucose sensitivity (de Courten et al., 2016). It is important to highlight, that many of these purported benefits are based on animal, or *in vitro*, models, and it is yet to be determined whether BA supplementation can impact these processes and conditions. As such, further research is warranted to confirm the therapeutic or clinical efficacy of BA supplementation.

These wide-ranging proven, or purported, benefits of BA supplementation have created an ever-increasing market, and it is a very commonly used dietary supplement. But many questions remain open about the MCarn response to BA supplementation, and these questions must be addressed in order to optimize the efficacy and applicability of this nutritional strategy (Perim et al., 2019). It seems that substantial amounts of BA are required to increase MCarn, with most studies using doses of ∼3.2–6.4 g·day^{-1}, for periods ranging from 4 to 24 weeks. It is likely that these large amounts of BA are required because the incorporation of ingested BA into the muscle is very low, with ∼3–6% of ingested BA estimated to contribute toward MCarn accumulation (Stegen et al., 2013; Blancquaert et al., 2015). BA uptake into the muscle seems to be rapid and efficient, however the ability of carnosine synthase to incorporate it into MCarn is far slower (Bakardjiev and Bauer, 1994; de Souza Goncalves et al., 2020) and so the remaining BA is likely to be converted toward other processes such as transamination or oxidation (Blancquaert et al., 2016). Despite this inefficiency in the use of supplemental BA to synthesize MCarn, very large increases, to the order of 200%, have been reported (Saunders et al., 2017b). The capacity of the muscle to uptake and increase MCarn, and the quantity of BA required to achieve saturation is not, however, currently known. Inter-individual variability in response to supplementation seems to be high (Saunders et al., 2017b), yet little is known about what factors underpin this variation, nor what the individual proportion of response to BA supplementation actually is. Factors such as age, sex, and baseline carnosine content may all theoretically impact subsequent response to supplementation, although consideration of individual datasets indicates that this may not be the case (Baguet et al., 2012; Stellingwerff et al., 2012). To address these, and other, questions, we conducted a comprehensive analysis, comprising various modeling techniques, to synthesize existing understanding about the nature of the MCarn response to BA supplementation. We also analyzed individual participant data from studies conducted within our laboratory; and considered these findings within the context of published summary data, which was analyzed using a frequently used dose-response model (Emax). All analyses were conducted from a Bayesian perspective, the advantages of which is that it allowed the flexibility to use a range of models in order to more fully represent the available data, while simultaneously allowing the results to be interpreted intuitively and probabilistically (Dunson, 2001).

MATERIALS AND METHODS

The protocol for this study was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The Population, Intervention, Comparator, Outcomes and Study Design (PICOS) approach was used to guide the inclusion and exclusion of studies for this review and are described in **Table 1**. In addition to conducting a systematic search of available literature, we also combined all available individual data from studies conducted within the authors lab, all of which used a dosing strategy of 6.4 g·day^{-1}, for periods varying between 4 and 24 weeks. Muscle carnosine content was measured using HPLC analysis of muscle biopsy data, and the full protocol for this analysis is described elsewhere (Saunders et al., 2017b).

Search Strategy

The search strategy was based on a three-step screening (title/abstract screening, full-text screen and full text appraisal), independently undertaken by two reviewers. This search was originally conducted to inform a systematic risk assessment on the use of BA supplementation (Dolan et al., 2019b).

TABLE 1 | Study inclusion and exclusion criteria.

Population	Healthy individuals of any age or physical activity level.
Intervention	Original studies investigating the effects of oral BA supplementation on skeletal MCarn content.
Comparator	No human comparators were required in the studies included in this review, although the data from placebo groups were used to quantify biological variability across the time periods investigated, when available.
Outcomes	The primary outcome was the effect of BA supplementation on skeletal MCarn concentration. Potential moderators to this response included dose, sex, age, baseline MCarn, and the method used to measure MCarn.
Study design	Controlled or uncontrolled intervention studies.

This risk assessment included all BA supplementation studies (including both human and animal models). One hundred and one human studies were included in that investigation and were subsequently screened to identify those that included an MCarn measurement. The protocol for that review was prospectively registered (PROSPERO registration no. CRD42017071843).

Data Analysis

The present study comprised both individual and aggregate data meta-analyses from a Bayesian perspective. Individual data were pooled using mixed effects multilevel models. Analyses were performed on the outcome variable MCarn (absolute value) to quantify the effects of BA supplementation and random noise due to biological variation and measurement error. Additionally, proportion of response was estimated across controlled studies by calculating inter-individual difference in response to supplementation and comparing this to a non-zero increase in MCarn (Swinton et al., 2018). Bayesian estimates of the standard deviation in observed change from active and placebo groups were used to obtain the intervention response standard deviation (σ^,_IR) describing inter-individual difference in response. Aggregate data meta-analyses were performed using published pre- and post-intervention mean and standard deviation values. Values were transformed into standardized mean differences (SMD) and sampling variance calculated using methods described previously (Saunders et al., 2017a). Three-level mixed effects models were used to quantify the effects of supplementation dose. Insufficient data were available to allow investigation of the interaction between daily dose and intervention duration and so the total cumulative dose ingested was selected as the primary outcome, which previous research has identified as being more influential than either daily dose or intervention duration (Stellingwerff et al., 2012; Church et al., 2017; Dolan et al., 2019b). Subset analyses using study covariates were used to assess the effects of sex, age, or measurement method on the main effect of BA supplementation. Finally, a model-based approach was employed to investigate the dose-response relationship between cumulative BA supplementation and the SMD. A standard four parameter sigmoid predicted maximum effect (Emax) model was estimated with:

$$E = E_0 + \frac{E_{\max} \times C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}$$

Where E is the effect size (SMD), E_0 is the baseline effect, E_max is the maximum effect, EC_50 is the cumulative dose that provides 50% of the maximum effect, C is the input (cumulative dose) and γ is the Hill coefficient controlling the slope of the sigmoid response. Inferences from all models were performed on posterior samples generated by Markov Chain Monte Carlo with Bayesian 95% credible intervals (CrIs) constructed to enable probabilistic interpretations of parameter values. Models were run in OpenBUGS (version 3.2.3, MRC Biostatistics Unit) and in R (version 3.3.1 R Development Core Team) using the R2OpenBugs package.

RESULTS

Group Study Characteristics

Twenty-six studies were identified in the systematic search and included in the meta-analysis (Harris et al., 2006, 2010; Derave et al., 2007; Hill et al., 2007; Kendrick et al., 2008, 2009; Baguet et al., 2009, 2010; del Favero et al., 2012; Stellingwerff et al., 2012; Stegen et al., 2013; Bex et al., 2014, 2015; Chung et al., 2014; Danaher et al., 2014; Gross et al., 2014; Kresta et al., 2014; Cochran et al., 2015; Blancquaert et al., 2017; Church et al., 2017; Saunders et al., 2017b; Varanoske et al., 2017, 2018; Black et al., 2018; Carvalho et al., 2018; da Eira Silva et al., 2020). We also included data from two other, currently unpublished, studies conducted within the authors lab. These studies met all of the inclusion criteria described herein. The decision to include them was based on the additional power that the increased sample brought, as well as to ensure that the statistical analysis generated the best possible estimate of the true value (see **Figure 1** for search flow diagram). In total, 575 participants (comprising 486 men and 89 women) were included in the meta-analysis, of which 382 consumed BA, with the remaining 193 allocated to a placebo intervention. The majority of studies were conducted on healthy young adults (mean age (yrs) = 23.89, SD = 5.46), with only one study conducted on older adults [mean age (yrs) = 64.34, SD = 4.99 (del Favero et al., 2012)] and none on younger adults (<18 years). An overview of all included studies is presented in **Supplemental Table 1**. Analyses were completed on subsets of the data depending on the specific analysis and suitability of each study set, as described below.

Individual Data

Complete individual data sets were available for 99 participants (BAn = 67, PLAn = 32) comprising a total of 232 observations, some of which was previously published (Saunders et al., 2017b; Carvalho et al., 2018; da Eira Silva et al., 2020). All studies were conducted on young men and provided a BA dose of 6.4 g·day^{-1} with observations ranging from 4 to 24 weeks post baseline. BA supplementation increased MCarn on average by 16.0 mmol·kgDM^{-1} [95%CrI: 12.4–19.6] compared to placebo. Regression analyses with duration centered at 4 weeks were completed to determine if the effects of supplementation

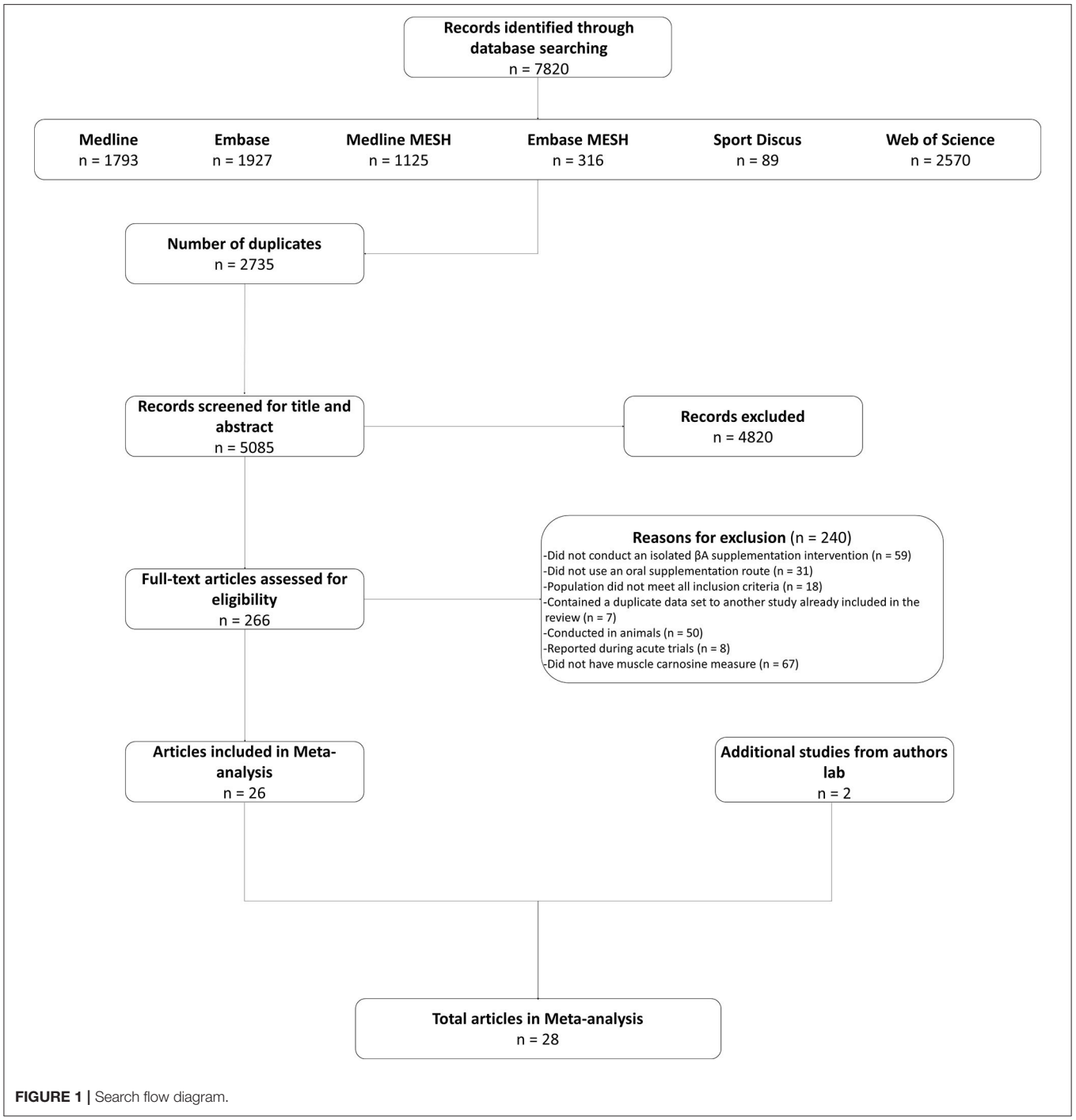

FIGURE 1 | Search flow diagram.

increased beyond this point (BAn = 50, 134 total observations). The mean change in MCarn at 4 weeks was 14.0 mmol·kgDM^{-1} [95%CrI: 10.1–18.1], with a positive regression slope indicating a further 0.5 [95%CrI: 0.2–0.7] mmol·kgDM^{-1} increase per week. Analyses of the same data also demonstrated that baseline levels of MCarn were not associated with changes due to supplementation (−0.1 [95%CrI: −0.3–0.1]). The amount of random noise in MCarn values due to biological variation and measurement error (i.e., typical variation) was estimated using observations from placebo groups. The standard deviation of residuals from the multilevel model representing typical variation was 4.1 mmol·kgDM^{-1} ([95%CrI: 3.4–5.1], PLAn = 18, 61 total observations). The intervention response standard deviation ($\sigma^{\wedge}$,_IR) was estimated as 6.6 mmol·kgDM^{-1} [95%CrI: 3.4–9.4] and the proportion of individual response was 99.3% [95%CrI: 96.2–100].

Aggregate Data

Aggregate analyses were based on effect sizes calculated from all available studies using the SMD pre to post change in MCarn levels. One hundred and eight effect sizes were available from BA groups only, six of which were removed as they were outliers (ES > 5). The multilevel meta-analysis with no study covariates estimated a large pooled effect size of 1.5 [95%CrI 1.2–1.8], with substantial between ($\tau\hat{}20.5 = 0.6$) and within ($\epsilon\hat{}20.5 = 0.7$) study variance (**Figure 2**). The same model applied to effect sizes calculated with supplementation and control group data (22 studies and 56 effect sizes) also produced a large pooled effect size of 1.7 [95%CrI: 1.3–2.1], with substantial between ($\tau\hat{}20.5 = 0.8$) and within ($\epsilon\hat{}20.5 = 0.5$) study variance (**Figure 3**). Using a simple linear model, the effects of cumulative BA dose was assessed by centering on the mean value (208 g). Results demonstrated a large effect at the mean cumulative dose (1.5 [95%CrI: 1.2–1.8]) and an estimated 0.23 [95%CrI: 0.06–0.49] increase in effect size per additional 100 g. Similar results were obtained for effect sizes calculated with supplementation and control group data (effect at mean: 1.7 [95%CrI: 1.3–2.1]; effect per additional 100 g: 0.16 [95%CrI: 0.01–0.31]). Insufficient data were available to ascertain if age altered the effects of BA supplementation, but subset analyses were conducted to investigate the impact of sex and the method used to measure MCarn, using effect sizes generated from supplementation groups only. Sixteen studies were selected that used the most common dosing protocol (cumulative dose between 130 and 180 g) comprising a total of 56 effect sizes. For the sex comparison there were eight effect sizes from a female only group, 38 effect sizes from a male only group and 10 effect sizes from a mixed group. No substantive evidence of an effect of sex was obtained (male vs. female: −0.32 [95%CrI: −1.1–0.43]; male vs. mixed: −0.00 [95%CrI: −0.95–0.88]). Across the 16 studies, 40 effect sizes were obtained from MCarn values measured with non-invasive scanning devices (i.e., HR-MRS) and 16 effect sizes obtained with muscle biopsy based analyses (mainly assessed by HPLC, with one study using UPLC and one using mass spectrometry), with some evidence of increased effects with HPLC (0.16 [95%CrI: 0.01–0.43]).

Emax Model

The predicted maximum effect of BA supplementation (Emax) was 3.0 [50%CrI: 2.2–3.7] and the estimated total cumulative dose (g) required to achieve 50% of this maximum effect (ED50) was 377 g [50%CrI: 210–494]. A density plot with the Emax curve generated from median parameter values is provided in **Figure 4**. An extrapolation of posterior samples from the Emax model was performed to estimate probabilities that percentage of maximum effect could be achieved with cumulative doses ranging from 1,000 to 1,500 g (see **Table 2**). These results estimated, for example, that the probability of obtaining at least 70% of maximum effect with a cumulative dose of 1,000 g was 0.68.

DISCUSSION

The purpose of this study was to conduct a comprehensive analysis with various modeling techniques to synthesize existing knowledge about the MCarn response to BA supplementation. Collectively, our findings, based on all models employed, indicated that human skeletal muscle has large capacity for MCarn accumulation, and that commonly used protocols (e.g., 4 weeks at 6.4 g·day^{-1}) may not come close to saturating MCarn. Baseline values do not appear to influence subsequent response to supplementation and the non-linear response to supplementation was not influenced by sex. Analysis of individual data indicate that MCarn is relatively stable in the absence of intervention, and that effectually all (99.3% [95%CrI: 96.2–100]) participants respond to BA supplementation.

Our analyses indicate humans have large capacity for non-linear MCarn accumulation in response to BA supplementation. **Figure 4** shows that BA supplementation can lead to a maximum effect size of ∼3. Take, for example, the individual data set used in the current analysis, which had a baseline mean ± SD MCarn of 22.9 ± 8.7 mmol·kgDM^{-1}. Intake of 1,500 g of BA is estimated to lead to an approximate increase of three times this standard deviation, (i.e., ∼26.1 mmol·kgDM^{-1}). It is important to highlight that these estimates are based on the median expected effect, and considerable inter-individual variation is likely. Additionally, estimates at the higher end of the curve described in **Figure 4** should be interpreted with caution, as a paucity of data based on very high doses limits precision regarding the point at which human skeletal muscle saturation occurs. Despite these caveats, our data provides new insight into the nature of the MCarn response to BA supplementation, and how this differs to other commonly used dietary supplements, such as creatine. Human skeletal muscle appears to reach creatine saturation at ∼140–160 mmol·kgDM^{-1} (Harris et al., 1992) and this can be achieved within 5 days of high-dose supplementation. Response to creatine supplementation is largest in those with lowest baseline levels, whereas individuals whose creatine content is habitually closer to this saturation point gain smaller benefit from supplementation (Harris et al., 1992). In contrast, we observed no evidence that baseline MCarn influenced response to supplementation. This makes sense when considered in relation to our predictive model, as it seems that humans have large capacity to accumulate MCarn—far greater than is achieved with commonly used protocols (e.g., 179.2 grams provided as 6.4 g·day^{-1} for 4 weeks). This may be because baseline MCarn contents (∼25 mmol·kgDM^{-1}) are substantially lower than predicted maximum capacity, whereas humans seem to habitually maintain creatine content at levels far closer to the proposed creatine saturation limit of ∼140–160 mmol·kgDM^{-1}.

Our model indicates that MCarn increase in response to BA supplementation is non-linear, and that the greatest increases occur in the earlier stages of supplementation. This finding aligns with a recent theoretical model proposed by Spelnikov and Harris (2019), which describes absolute MCarn increases as a product of both synthesis and decay, with carnosine synthesis considered to be constant in relation to time and first order to daily BA dose. Similarly, carnosine decay is also considered to be first order, but to relate to total MCarn content. As such, carnosine decay increases when absolute content is higher and so the rate of MCarn accumulation due to BA induced elevations in synthesis will slow, as illustrated in **Figure 4**. Tissue saturation represents

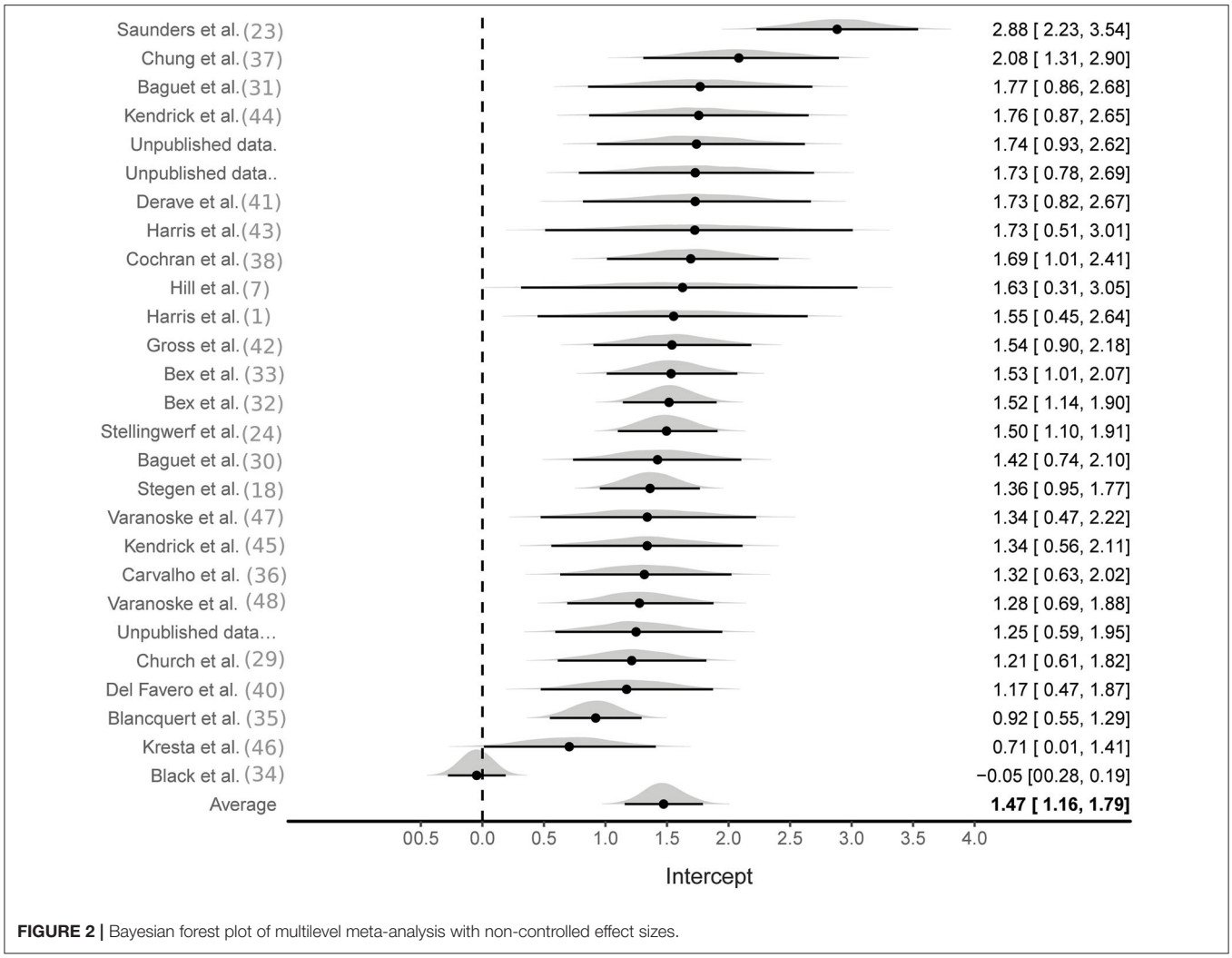

FIGURE 2 | Bayesian forest plot of multilevel meta-analysis with non-controlled effect sizes.

the point at which the rates of synthesis match decay, and so content remains constant despite continued supplementation. The exact point, and nature, of this saturation point is not currently known. Does human skeletal muscle have a largely uniform saturation point, after which no further increases can be attained (as seems to be the case with creatine)? Or does capacity to accumulate MCarn vary widely between individuals, with each having their own upper limit? Currently, insufficient data using very high BA protocols on MCarn precludes the answering of this question, but one thing that is clear is that human skeletal muscle has large capacity to uptake BA and to increase MCarn, and that in the absence of intervention, MCarn is maintained at levels far below its maximal capacity.

The Emax model illustrated in **Figure 4** clearly shows that very large amounts of BA are required to reach MCarn saturation. Theoretically, the greater the increase in MCarn content, the greater its ability to buffer, and to contribute to other processes such as anti-oxidation and anti-glycation, and so intuitively, attaining the largest increases possible seems desirable. But evidence on this hypothesis is conflicting. Two individual studies reported that larger MCarn increases were associated with greater performance effects (Hill et al., 2007; Saunders et al., 2017b), but this assertion is not supported by meta-analytic data, which indicates that the total dose ingested does not influence its effect on exercise performance (Saunders et al., 2017a). It would be counterintuitive to believe that performance benefits could linearly increase with ever-increasing MCarn, given that numerous factors, apart from acidosis, contribute to fatigue, and so it makes sense that at some point, performance benefits must plateau. Identification of the lowest MCarn increase necessary to elicit an ergogenic effect, along with the point after which no further benefits can be obtained would have large potential to enhance the applicability and efficacy of BA supplementation strategies. For example, it seems that the largest gains in MCarn are attained in the earlier phases of supplementation (see **Figure 4**). It would be of interest to identify if strategies such as meal co-ingestion (Stegen et al., 2013), intake in proximity to training (Bex et al., 2015), or intake in slow-release capsules (Varanoske et al., 2018) can influence the early response to supplementation (Perim

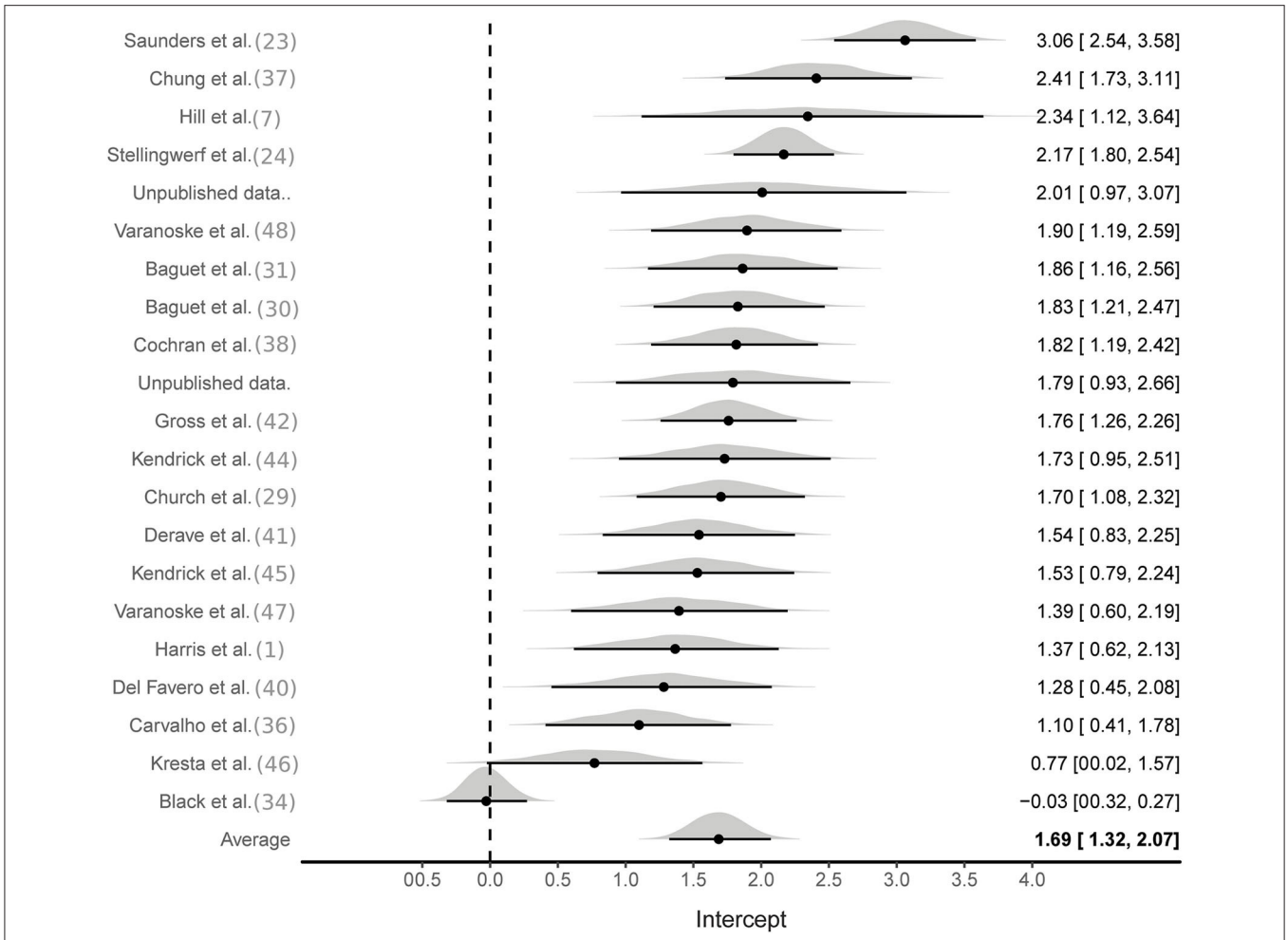

FIGURE 3 | Bayesian forest plot of multilevel meta-analysis with controlled effect sizes.

et al., 2019) and whether this, in turn, meaningfully impacts exercise performance.

In addition to investigating whether or not greater MCarn increases are likely to bring about greater benefits, it is also important to weigh up the potential cons, against the potential pros, of this approach. From a practical point of view, dosing protocols of the magnitude required to cause saturation would be challenging. Additionally, BA supplementation in its current doses is regarded as having no adverse effects (Dolan et al., 2019b), but it is unknown if this will remain true at the substantially higher doses that are apparently required to reach saturation. Paresthesia, which is commonly described as a "pricking" or "tingling" sensation, commonly occurs during BA supplementation, likely due to the binding of BA to the peripheral neuronal receptor MrgprD (Liu et al., 2012). This sensation is not considered to be harmful but may be deemed unpleasant by some individuals. Paresthesia intensity is related to the timing of peak blood BA concentrations (Harris et al., 2006) and it is possible that large dosing increases, of the magnitude predicted to be necessary to achieve MCarn saturation, may invoke sensations deemed intolerable. Another theoretical adverse effect of prolonged BA supplementation is a decrease in taurine content, given that the two share a transporter (Tau-T) (Shaffer and Kocsis, 1981). We have previously reported that very high BA doses (namely those commonly used in animal trials) result in a substantial depletion of intracellular taurine (Dolan et al., 2019b) but the same does not hold true for human studies (Dolan et al., 2019b; Saunders et al., 2020), likely due to the substantially lower doses typically employed (Dolan et al., 2019b). It is possible that the very high doses apparently required for MCarn saturation, may lead to taurine reductions, and so some caution must be taken in attempting to implement substantially higher doses than those currently in use. Similarly, previous research highlighted that L-histidine is also required for carnosine synthesis, and that chronic BA supplementation may cause depletion of the free histidine pool, which in itself may have implications given the wide range of physiological processes that histidine contributes to Blancquaert et al. (2017). Similar to that which was observed for taurine, meta-analytic data indicated that BA dosing protocols within the ranges commonly used do not impact the free histidine

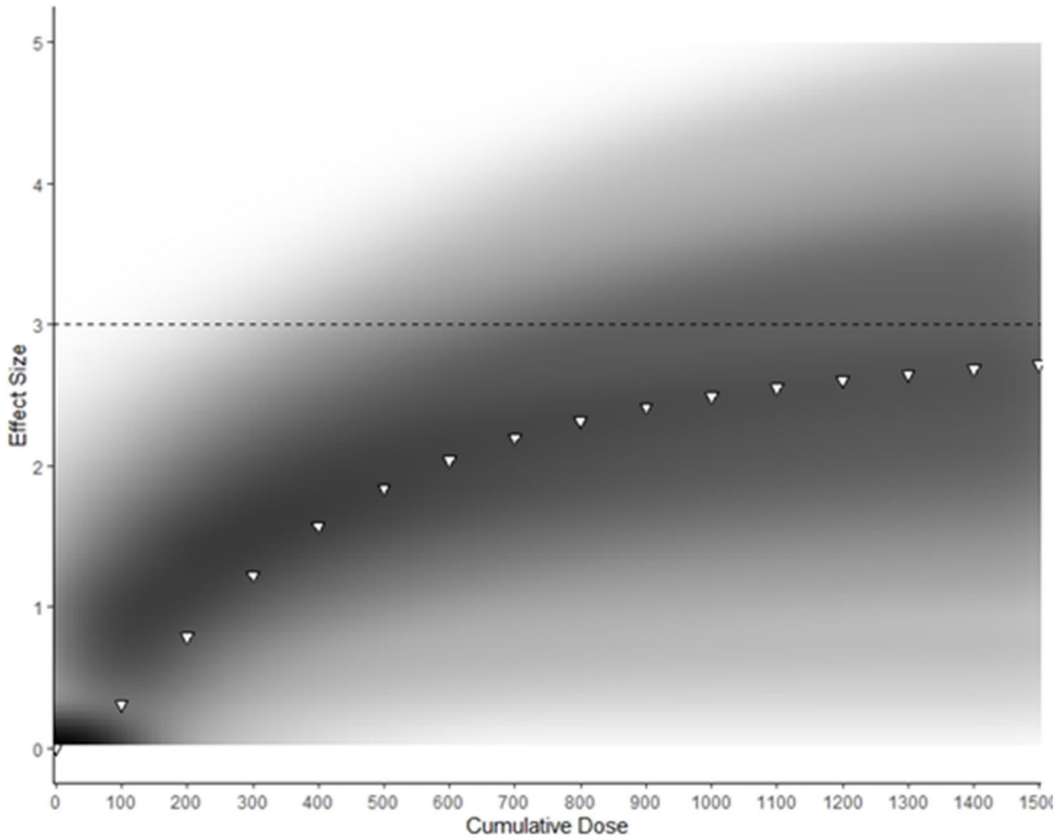

FIGURE 4 | Density plot of Bayesian Emax model predicting effect of cumulative BA supplementation on muscle carnosine content. Darker areas represent more common Emax trajectories. White triangles represent Emax generated with median parameter values. The dotted line represents the predicted maximum effect of BA supplementation on MCarn.

pool (Dolan et al., 2019b), however no evidence currently exists to indicate whether or not this would remain true in the event of substantially increased BA dosing protocols. Collectively, the available evidence indicates that achieving the very high MCarn levels that the current Emax model indicates are possible, but may not be desirable, due to practical and safety issues. We suggest that in lieu of investigating means of maximizing intracellular carnosine content, future research efforts should instead focus on the point at which maximum ergogenic benefits are attained, as well as the point after which no further ergogenicity occurs.

The current analysis also brought to light some interesting points about the nature of the MCarn response to supplementation, which has implications for future study design. In the absence of intervention, MCarn seems to be relatively stable, likely due to low intramuscular carnosinase and roughly equivalent synthesis and degradation rates (Boldyrev et al., 2013). Our analysis of individual data indicated typical variation of ∼4 mmol·kgDM^{-1} across a 4-weeks period. Reliability data indicate that two muscle samples taken from the same biopsy cut vary by ∼1 mmol·kgDM^{-1}, and so measurement error likely accounts for at least a quarter of this variation, and probably a lot more given that this estimate was based on samples taken moments apart and from the same biopsy cut. Interestingly, both within and between study variance were large and similar. A large proportion of this sampling error is likely due to small sample sizes. Typically, the use of a control group would be recommended to normalize the effects of the intervention against those of usual biological variability (Swinton et al., 2018). But in this situation, we observed little variation in placebo group MCarn, while the effects of intervention studies when analyzed both with, and without, controlling for the effects of the placebo group were similar (ES [95%CrI]: 1.7 [1.3–2.1] vs. 1.5 [1.2–1.8]). This implies that the control group adds little value to the analysis, likely because of MCarn stability and the large effect of supplementation. In future investigations of the MCarn response to BA in young healthy males (and particularly those for which resources are limited) it may be prudent to direct resources toward the intervention group, in order to reduce within study variance. It is important to note that this recommendation applies only to studies on the MCarn response to BA supplementation. The influence of BA supplementation on exercise performance, or clinical outcomes, is far less well-characterized and subject to substantially more sources of internal and external variability

TABLE 2 | Probability table representing the chance that various cumulative doses (columns) create a response greater than the specified percentage of EMax (rows) based on Bayesian model generated.

% EMax	1,000 g	1,100 g	1,200 g	1,300 g	1,400 g	1,500 g
70%	0.68	0.73	0.77	0.80	0.83	0.85
80%	0.45	0.48	0.51	0.54	0.56	0.59
90%	0.31	0.33	0.35	0.37	0.38	0.40

Values in table represent probabilities (p) $0 \leq p \leq 1$.

and so control groups are essential in studies for which exercise, or clinical effectiveness, is the primary outcome of interest.

In addition to characterizing the nature of MCarn response to BA supplementation, we also considered the influence of various potential moderators on this response. In relation to the method of assessment, it seems that lower effect estimates are generally observed when MCarn is measured using the H-MRS technique when compared to those obtained using HPLC analysis of muscle biopsies. Only one study showed no MCarn increase, despite using a commonly used dosing protocol of 6.4 g·day^{-1} for 28 days (Black et al., 2018). It is important to highlight that the MRS measurements reported in that study used a 1.5T magnet, as opposed to all others which used a 3T magnet. Given the incongruency of this finding in comparison to all others, it seems plausible that this may have occurred due to methodological inadequacies. When considering the influence of non-modifiable factors on the MCarn response to supplementation (namely age and sex), we could not conduct analyses on the influence of age, as insufficient data in older groups, and no data on younger groups, were available. Further research investigating the influence of BA supplementation on MCarn in older adults, along with potential therapeutic or ergogenic benefits, would be of interest, although it is worth highlighting that the one study that investigated a group aged 60–80 years did show comparable increases to other studies conducted in younger populations (del Favero et al., 2012). Women have previously been reported to have lower MCarn than men (Mannion et al., 1992; Everaert et al., 2011), which may be due to factors such as gender dimorphism in sex steroid concentrations (Peñafiel et al., 2004) or to variation in fiber-type composition (Dunnett et al., 1997; Hill et al., 2007; Painelli et al., 2018). Despite these differences, our data indicate that both men and women have a similar response to BA supplementation, indicating that the lower values previously reported in women are unlikely to relate to an inherent gender dysmorphism in the biological factors that underpin carnosine metabolism.

In conclusion, our findings indicate that human skeletal muscle has large capacity to accumulate carnosine. MCarn remains stable in the absence of intervention and neither low baseline MCarn levels, nor sex, influence the subsequent response to BA supplementation. In turn, these findings lead to other questions, the response to which may have large implications for future practice. From the point of view of athletic performance, key questions include: what is the absolute MCarn increase required to elicit an ergogenic effect, along with the point after which no further benefits are attained? It is clear that 4 weeks of BA supplementation can be ergogenic, but can this be achieved earlier? Can strategies to enhance the early response to BA supplementation meaningfully impact the subsequent ergogenic benefits? The response to these questions may progress practical application of this supplementation strategy, with potential benefit to many athletic and clinical populations.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**. Any additional information is available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

ED, PS, BS, and BG designed the research. ED and NR conducted the searches. NR, LO, and RS extracted all data. KN, RS, GY, BS, and VE collected all original data used in the individual analysis. ED and NR wrote the manuscript, with ongoing critical input from PS, BG, GA, and BS. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00913/full#supplementary-material

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Sodium Bicarbonate Ingestion Improves Time-to-Exhaustion Cycling Performance and Alters Estimated Energy System Contribution: A Dose-Response Investigation

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This study investigated the effects of two sodium bicarbonate ($NaHCO_3$) doses on estimated energy system contribution and performance during an intermittent high-intensity cycling test (HICT), and time-to-exhaustion (TTE) exercise. Twelve healthy males (stature: 1.75 ± 0.08 m; body mass: 67.5 ± 6.3 kg; age: 21.0 ± 1.4 years; maximal oxygen consumption: 45.1 ± 7.0 ml.kg.min^{-1}) attended four separate laboratory visits. Maximal aerobic power (MAP) was identified from an incremental exercise test. During the three experimental visits, participants ingested either 0.2 g.kg^{-1} BM $NaHCO_3$ (SBC2), 0.3 g.kg^{-1} BM $NaHCO_3$ (SBC3), or 0.07 g.kg^{-1} BM sodium chloride (placebo; PLA) at 60 min pre-exercise. The HICT involved 3 × 60 s cycling bouts (90, 95, 100% MAP) interspersed with 90 s recovery, followed by TTE cycling at 105% MAP. Blood lactate was measured after each cycling bout to calculate estimates for glycolytic contribution to exercise. Gastrointestinal (GI) upset was quantified at baseline, 30 and 60 min post-ingestion, and 5 min post-exercise. Cycling TTE increased for SBC2 (+20.2 s; $p = 0.045$) and SBC3 (+31.9 s; $p = 0.004$) compared to PLA. Glycolytic contribution increased, albeit non-significantly, during the TTE protocol for SBC2 (+7.77 kJ; $p = 0.10$) and SBC3 (+7.95 kJ; $p = 0.07$) compared to PLA. GI upset was exacerbated post-exercise after SBC3 for nausea compared to SBC2 and PLA ($p < 0.05$), whilst SBC2 was not significantly different to PLA for any symptom ($p > 0.05$). Both $NaHCO_3$ doses enhanced cycling performance and glycolytic contribution, however, higher doses may maximize ergogenic benefits.

Keywords: anaerobic, ergogenic aid, high-intensity exercise, alkalosis, fatigue, extracellular buffer

INTRODUCTION

High-intensity interval training (HIIT) involves near maximal exercise bouts (>80–100% maximum heart rate) separated by brief recovery periods (1). The high anaerobic demand associated with maximal efforts results in the accumulation of hydrogen cations (H^+) within the cytosol (2). Whilst these are mostly removed by intramuscular and/or extracellular buffering mechanics, production overwhelms neutralization, and this contributes toward a reduced intramuscular pH (3), causing exercise-induced acidosis. Such a biochemical state has been suggested to reduce glycolytic energy production and may disrupt calcium ion cross-bridge formation (4). A common strategy to mitigate these deleterious effects of exercise is to enhance circulating level of extracellular blood bicarbonate (HCO_3^-), which subsequently allows for sustained efflux of H^+ from intramuscular environments during high-intensity exercise (5). Increases in [HCO_3^-] of ∼5.0–6.0 mmol.l^{-1} are suggested to be ergogenic and can be achieved via the ingestion of extracellular buffers, such as sodium bicarbonate ($NaHCO_3$) in doses of 0.2–0.3 g.kg^{-1} BM, respectively (6, 7).

Common practice is to ingest 0.3 g.kg^{-1} BM $NaHCO_3$ at 60–90 min prior to exercise, which is based on historical research showing time to peak pH or HCO_3^- occurs at this time point at the group mean level (6, 8). It is, however, likely that through following this strategy the dissociation of $NaHCO_3$ within stomach acid will cause gastrointestinal (GI) upset (9), which may impair performance or dissuade athletes from using $NaHCO_3$ (10, 11). Whilst, some authors have observed ergogenic benefits despite moderate GI upset (12, 13), in some cases the upset has been severe or the participant has not been able to continue with the study procedures (14, 15). The administration of smaller $NaHCO_3$ doses (0.2 g.kg^{-1} BM) might therefore be preferable, as it can mitigate GI upset and also reduce the sodium load per dose which might alleviate the health risks of ingesting this supplement; although these risks are more associated with long term use of $NaHCO_3$ (12, 16). McNaughton (17) reported exacerbated GI upset following higher $NaHCO_3$ doses, while Gough et al. (12) observed reduced occurrence of bowel urgency and bloating for 0.2 g.kg^{-1} compared to 0.3 g.kg^{-1} BM $NaHCO_3$. Reducing the dose is a simple strategy that might remove some of the negative connotations of ingesting this supplement, whilst it is far more cost effective than some of the recent strategies employed to reduce the GI upset following $NaHCO_3$ ingestion, such as in enteric-coated capsules (18, 19).

Contemporary research has administered $NaHCO_3$ using an individualized time-to-peak pH or HCO_3^- approach, which is in response to studies showing that time-to-peak pH or HCO_3^- can vary between 10 and 180 min within individuals, regardless of the ingestion method (i.e., capsule vs. fluid) (7, 12–14). In using the individual time-to-peak approach, this ensures that peak [HCO_3^-] is achieved immediately before exercise, which does seem to lead to a more consistent ergogenic response (12, 14). The identification of this time-to-peak HCO_3^- response presents a logistical challenge to athletes however, as the financial cost is high and requires specialist equipment and staff. It is plausible to suggest further research is therefore required to simplify this strategy, and to assess whether ergogenic benefits still exist for smaller $NaHCO_3$ doses following administration at a standardized time point. This, in turn, could increase the practical application of this supplement, whilst also potentially limiting GI upset.

The ergogenic benefits associated with $NaHCO_3$ ingestion are somewhat related to the increased activation of glycolytic energy pathways (20, 21). Whilst this is debated (22), $NaHCO_3$ ingestion attenuates muscle acidosis during exercise thus preventing the allosteric inhibition of glycogen phosphorlyase and phosphofructokinase (5). This has been shown to increase estimated glycolytic contribution during HIIT protocols (20), while there is robust evidence suggesting enhanced glycolytic flux within the muscle (23). Strategies that elevate glycolytic energy system contribution may enhance exercise capacity during HIIT, however, research is yet to determine whether smaller $NaHCO_3$ doses elicit a similar physiological response.

The purpose of this study therefore was to investigate the effect of 0.2 and 0.3 g.kg^{-1} BM $NaHCO_3$ ingested at 60 min pre-exercise on estimated energy contribution during a high-intensity, interval cycling test (HICT), and time-to-exhaustion (TTE) cycling performance.

MATERIALS AND METHODS

Experimental Approach to the Problem

A block randomized, across subjects counterbalanced, single-blind, placebo-controlled, crossover experimental design was implemented for this study. Participants visited the laboratory on four separate occasions to complete an incremental exercise test, familiarization, and three experimental trials. All testing was conducted at the same time of day (± 2 h) to minimize the confounding effects of circadian rhythms on exercise performance (24). Participants arrived at the laboratory in a 3-h post-prandial state, having refrained from alcohol ingestion and vigorous exercise for 24 h prior. Maximal aerobic power (MAP) was determined from the incremental exercise test and used to prescribe the exercise intensities for the HICT and TTE cycling protocols (described below). Participants completed these exercise procedures for three experimental treatment arms: (a) 0.2 g.kg^{-1} BM $NaHCO_3$ (SBC2), (b) 0.3 g.kg^{-1} BM $NaHCO_3$ (SBC3), or (c) 0.07 g.kg^{-1} BM sodium chloride to ensure taste-matching (placebo; PLA) (12). Participants were instructed to maintain activity levels and dietary intake throughout the study, which were assessed via written logs. All experimental trials were separated by 7 days.

Participants

Twelve healthy males (stature: 1.75 ± 0.08 m; body mass: 67.5 ± 6.3 kg; age: 21.0 ± 1.4 years; maximal oxygen consumption: 45.1 ± 7.0 ml.kg.min^{-1}) volunteered for this study. All participants were recreationally active and completed at least 60 min of vigorous exercise per week. Participants were excluded if they had any history of hypertension (>140/80 mmHg), were currently

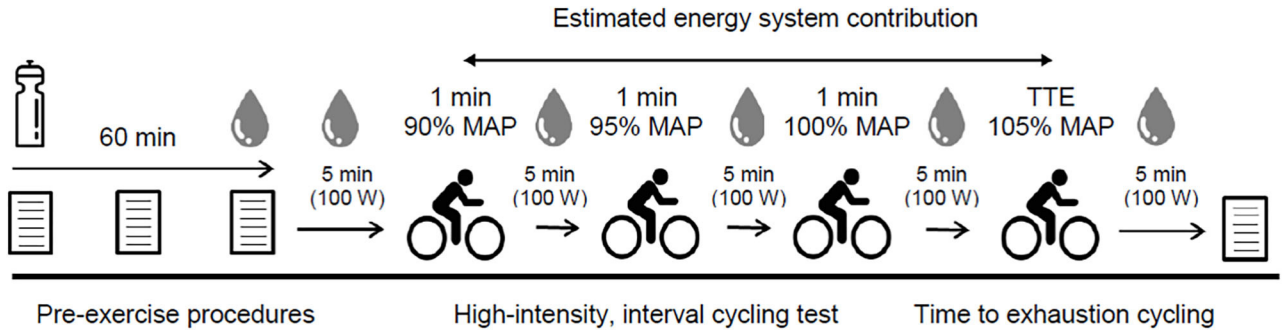

FIGURE 1 | Schematic overviewing procedures during experimental visits; MAP, maximal aerobic power; TTE, time to exhaustion.

taking any medication/sports supplements, or had ingested intra- or extracellular buffering agents within the previous 6 months. The study was approved by the institutional departmental review board. Each participant was informed of the benefits and risks of the investigation prior to signing informed consent to participate in the study. Procedures were conducted in accordance with the World Medical Association's Declaration of Helsinki.

Procedures

On the initial visit, participants performed an incremental exercise test on a cycle ergometer (Excalibur Sport, Lode, Netherlands) to determine MAP. Gaseous exchange was collected using a breath-by-breath metabolic cart (Oxycon Pro, Jaeger, Hoechberg, Germany) to determine maximal rate of oxygen consumption (VO_{2max}). To determine VO_{2max}, the highest 30 s rolling average was calculated. Following a 5-min warm-up (70 W; 70–90 rev.min^{-1}), increments of 20 W.min^{-1} were applied until volitional exhaustion. This was deemed as the failure to maintain cycling cadence >60 rev.min^{-1} despite verbal encouragement. Maximal anaerobic power was calculated as the fraction of time in the final stage divided by test increment, added to completed power (25). Familiarization to exercise procedures (HICT and TTE cycling) was completed after 30 min of passive recovery. This involved three bouts of 60 s cycling (90, 95, and 100% MAP), interspersed with 90 s of active recovery (100 W) and TTE cycling at 105% MAP. These were completed on the cycle ergometer, with handle bar and seat height position adjusted according to preference, which was subsequently replicated for all experimental trials. The TTE cycling protocol was terminated when cadence dropped 10 rev.min^{-1} below the preferred cadence, and when participants were unable to re-establish preferred cadence (range of selected cadence = 70–90 rev.min^{-1}). Participants were encouraged to exercise until volitional exhaustion, but total exercise time was not revealed.

During experimental trial visits, participants completed visual analog scales (VAS) were used for baseline GI upset (0 mm = "no symptom"; 100 mm = "severest symptom") that quantified the severity of nausea, flatulence, abdominal discomfort (AD), gut fullness (GF), bowel urgency rating (BUR), diarrhea, vomiting, and belching (12). Participants then consumed one of three experimental beverages (SBC2, SBC3, or PLA) across a 5-min period 60 min prior to exercise. Ingestion time was chosen in-line with previous work that showed the absorption kinetics between these doses are not significantly different up to this time point (14), and is the most practiced ingestion timing (6, 8). These were served as a chilled aqueous solution of 4 ml.kg^{-1} BM water and 1 ml.kg^{-1} BM squash (double strength orange squash, Tesco, UK) to increase the palatability and taste-match each beverage (26). A supplement belief questionnaire was completed post-ingestion to assess the efficacy of the single-blind design, and to ensure that no psychological bias regarding the impact of $NaHCO_3$ ingestion was transferred onto participants (27). Symptoms of GI upset were repeated at 30- and 60-min post-ingestion. Pre-exercise capillary blood samples were collected into 20 μL end-to-end sodium heparised capillary tubes (EKF Diagnostic GmbH, Germany) and analyzed for blood lactate concentration ([BLa^-]) using the Biosen C-Line (EKF Diagnostic GmbH, Germany). Participants rested for 5 min to determine baseline oxygen consumption and respiratory exchange ratio (RER), before completing the HICT and TTE protocols, during which gaseous exchange was measured throughout, and blood samples were taken after each cycling bout. Additional visual analog scales were completed immediately post-exercise for GI upset. An overview of experimental trials is displayed in **Figure 1**.

Estimated Energy System Contribution Calculations

Absolute energy demand and energy contribution from the oxidative and glycolytic energetic systems were estimated via non-invasive technique. The oxidative phosphorylation pathway (W_{AER}) was determined by subtracting resting oxygen consumption (i.e., the mean VO_2 value during the final 30 s of baseline) from the area under the oxygen consumption curve for each of the three 60 s bouts (90, 95, and 100% MAP) during the HICT (28). Area under the curve was calculated using the trapezoidal method. This approach has recently been shown to provide reliable and valid estimations for W_{AER} during intermittent exercise (20, 29). The glycolytic pathway ($W_{[LA]}$) was calculated from the assumption that a difference

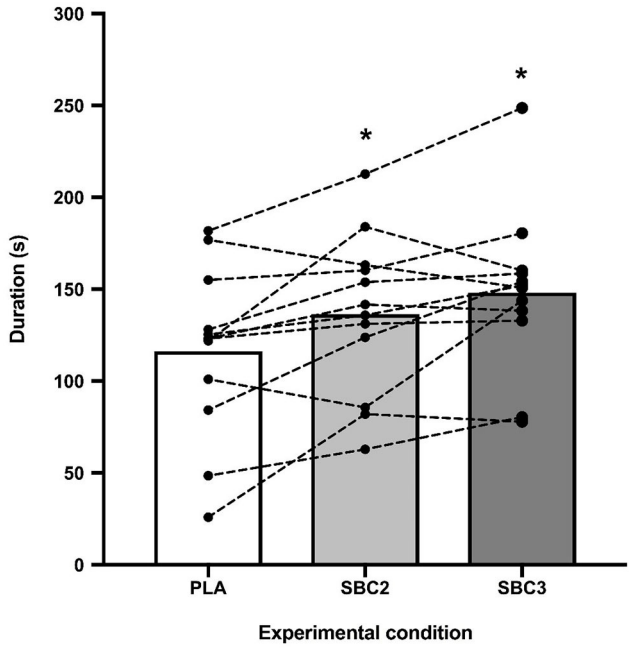

FIGURE 2 | Mean differences and inter-individual variation for TTE cycling performance; SBC2, 0.2 g.kg^{-1} BM $NaHCO_3$; SBC3, 0.3 g.kg^{-1} BM $NaHCO_3$; PLA, sodium chloride (placebo); * sig difference compared to PLA trial ($p < 0.05$).

of 1 mmol.l^{-1} of BLa^- obtained by subtracting baseline [BLa^-] from peak [BLa^-] (i.e., delta [BLa^-]) corresponded to 3 ml.kg^{-1} BM of O_2 (20, 29–32). Therefore, delta [BLa^-] for each of the three 60 s bouts and during TTE cycling (i.e., difference from pre to post) was multiplied by 3 and the participants' body mass to calculate $W_{[LA]}$. The caloric quotient of 20.92 kJ was used to convert between absolute energy demand (in L of O_2) and energy contribution (in kJ) for both energetic systems.

Statistical Analysis

Normality and sphericity were assessed using Shapiro-Wilk and Mauchly tests, before correcting for any violations (Greenhouse Geisser). One-way repeated measures analysis of variance (ANOVA) were conducted for cycling TTE performance and total energy demand and contribution from W_{AER} and $W_{[LA]}$ during exercise protocols. The smallest worthwhile change (SWC) in performance (9.1 s) was calculated as 0.3 x the between-individual SD for cycling TTE during familiarization (33). This was then used as a threshold for interpreting individual differences and in an attempt to identify a true change in exercise performance between the $NaHCO_3$ and the placebo conditions. Two-factor (treatment x time) repeated measures ANOVA's were performed for [BLa^-], RER, W_{AER}, and $W_{[LA]}$ for each of the three 60 s bouts during the HICT. When significant interactions were observed, pairwise comparisons using the bonferroni correction factor were performed. Friedman's two-way ANOVA's were conducted for GI upset. *Post-hoc* Wilcoxon matched-pair signed rank tests were performed when significance was observed, with median, Z score, and significance reported. Fisher's exact test was used to assess the efficacy of the single-blind design. For ANOVA interactions, effect sizes were presented as partial eta-squared (η_p^2) (34). Between treatment effect sizes were calculated by dividing the difference in means by the pooled SD (35), before applying a Hedges g (g) bias correction to account for the small sample size (36). These were interpreted as trivial (<0.20), small (0.20–0.49), moderate (0.50–0.79), or large (≥0.80) (37). Data are presented as mean ± SD and 95% confidence intervals (CI) reported for mean differences. Statistical significance was set at $p < 0.05$ and data were analyzed using SPSS v25 (SPSS Inc., IBM, USA).

RESULTS

Performance was greater for SBC2 (136.4 ± 43.5 s) and SBC3 (158.7 ± 63.3 s) compared to PLA (116.2 ± 46.6 s) (**Figure 2**). These increases were significant for SBC2 (+20.2 s; CI: 0.4, 39.9; $p = 0.045$; $g = 0.77$) and SBC3 (+31.9 s; CI: 10.8, 53.1; $p = 0.004$; $g = 1.13$). A total of 8 out of 12 participants improved their performance above the SWC following SBC2, whilst 11 participants (out of 12) improved above this threshold following SBC3 (**Figure 3**). There was an 11.7 s mean difference in favor of SBC3 vs. SBC2, but this increase was not significant ($p = 0.303$; $g = 0.48$). Nonetheless, seven of the participants (out of 12) improved their performance above the SWC for SBC3 vs. SBC2, whilst this was only in favor of SBC2 for a single participant.

Grouped mean ± SD data for [BLa^-] and RER are presented in **Table 1**. No significant differences were displayed during the HICT protocol ($p > 0.05$). Post-TTE [BLa^-] was elevated for SBC2 (+2.35 mmol.l^{-1}; CI: 0.06, 4.64; $p = 0.04$; $g = 0.77$) and SBC3 (+3.13 mmol.l^{-1}; CI: 1.44, 4.82; $p = 0.001$; $g = 1.40$) compared to PLA. There was a small effect size for SBC3 vs. SBC2 (+0.78 mmol.l^{-1}; $p = 0.34$; $g = 0.46$). Peak RER was also increased for SBC2 (+0.09 AU; CI: 0.03, 0.15; $p = 0.005$; $g = 1.14$) and SBC3 (+0.11 AU; CI: 0.03, 0.19; $p = 0.011$; $g = 0.98$) compared to PLA.

Total energy demand and contribution of the oxidative and glycolytic energetic systems during the HICT are presented in **Table 2**. No significant differences were displayed for energy demand or contribution from W_{AER} or $W_{[LA]}$ ($p > 0.05$), although $W_{[LA]}$ contribution was moderately increased for SBC2 (+3.71 kJ; $p = 0.09$; $g = 0.66$) and SBC3 (+7.12 kJ; $p = 0.14$; $g = 0.60$) compared to PLA (23.40 ± 8.93 kJ). There was a small effect size for $W_{[LA]}$ contribution when comparing SBC3 vs. SBC2 (+3.41 kJ; $p = 0.99$; $g = 0.27$). Energy contribution from W_{AER} was greater during the second 60 s bout for PLA vs. SBC2 (+4.16 kJ; CI: 0.50, 7.81; $p = 0.03$; $g = 0.86$). No significant differences were observed for energy contribution from W_{AER} or $W_{[LA]}$ during TTE cycling ($p > 0.05$; **Figures 4A,B**), although $W_{[LA]}$ was moderately increased for SBC2 (+7.77 kJ; $p = 0.10$; $g = 0.65$) and SBC3 (+7.95 kJ; $p = 0.07$; $g = 0.70$) compared

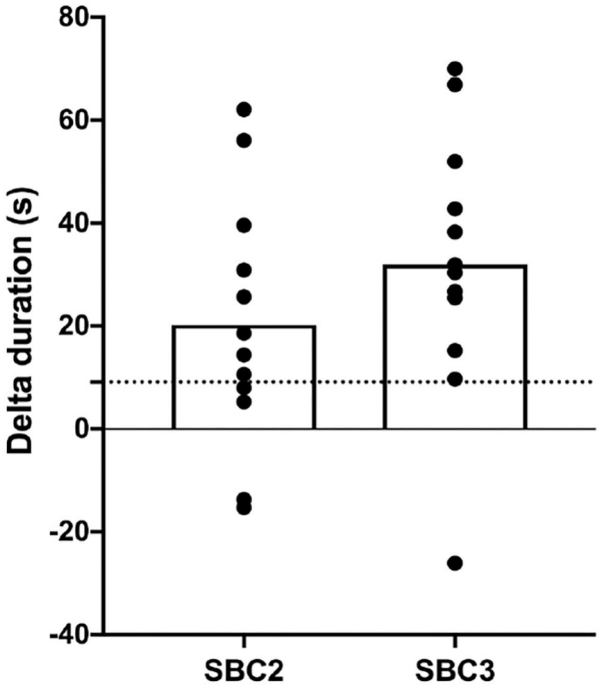

FIGURE 3 | Individual changes (with mean; clear bar) in TTE duration compared to PLA condition; SBC2, 0.2 g.kg^{-1} BM $NaHCO_3$; SBC3, 0.3 g.kg^{-1} BM $NaHCO_3$; PLA, sodium chloride (placebo); dashed horizontal line depicts SWC in performance (9.1 s).

TABLE 1 | Physiological variables ([BLa$^-$] and RER) obtained during the HICT and TTE cycling.

		90%	95%	100%	TTE
[BLa$^-$] (mmol.l^{-1})	SBC2	4.71 ± 1.38	6.91 ± 1.52	8.73 ± 1.80	14.09 ± 3.95*
	SBC3	4.30 ± 1.43	6.86 ± 1.66	9.35 ± 3.68	14.87 ± 3.01*
	PLA	4.26 ± 1.43	6.79 ± 2.06	8.13 ± 2.46	11.74 ± 3.47
RER (AU)	SBC2	1.08 ± 0.06	1.07 ± 0.04	1.08 ± 0.03	1.25 ± 0.06*
	SBC3	1.11 ± 0.05	1.09 ± 0.06	1.09 ± 0.06	1.26 ± 0.10*
	PLA	1.08 ± 0.07	1.07 ± 0.06	1.05 ± 0.04	1.15 ± 0.06

Data are mean ± SD; HICT, high-intensity, interval cycling test (60 s bouts at 90, 95, and 100% maximal aerobic power); TTE, time to exhaustion; SBC2, 0.2 g.kg^{-1} BM $NaHCO_3$; SBC3, 0.3 g.kg^{-1} BM $NaHCO_3$; PLA, sodium chloride (placebo).
** sig difference compared to PLA ($p < 0.05$).*

to PLA (15.62 ± 9.27 kJ). No difference was reported for $W_{[LA]}$ when comparing SBC3 vs. SBC2 (+0.18 kJ; $p = 1.00$; $g = 0.01$).

Treatments were successfully single-blinded and taste-matched (Fisher's exact test, $p = 0.28$). One subject identified all three beverages, eight only correctly perceived one of the three beverages, and the remaining three were unsure on all treatments. Eight participants reported their severest symptom after either SBC2 (4/12) or SBC3 (4/12), although some reported no difference between treatments (3/12), whereas one experienced

TABLE 2 | Total energy demand and contribution of the oxidative and glycolytic systems during the HICT.

		SBC2	SBC3	PLA
Energy demand (L of O_2)	W_{AER}	5.1 ± 0.9	5.1 ± 0.8	5.3 ± 0.8
	$W_{[LA]}$	1.3 ± 0.4	1.5 ± 0.8	1.1 ± 0.4
Energy contribution (kJ)	W_{AER}	105.8 ± 18.9	106.4 ± 17.0	110.1 ± 17.2
	$W_{[LA]}$	27.1 ± 8.5	30.5 ± 17.4	23.4 ± 8.9

Data are mean ± SD; HICT, high-intensity, interval cycling test (60 s bouts at 90, 95, and 100% maximal aerobic power); W_{AER}, oxidative phosphorylation contribution; $W_{[LA]}$, glycolytic contribution; SBC2, 0.2 g.kg^{-1} BM $NaHCO_3$; SBC3, 0.3 g.kg^{-1} BM $NaHCO_3$; PLA, sodium chloride (placebo).

the severest symptom following PLA (**Table 3**). No intervention or time interaction was observed at 30- or 60-min post-ingestion for any GI symptom ($p > 0.05$), or at post-exercise for vomiting, flatulence, GF, BUR, or diarrhea ($p > 0.05$). Nonetheless, symptom severity was increased post-exercise following SBC3 compared to PLA for nausea (10.0 vs. 1.0 mm; $Z = -2.197$; $p = 0.028$) and belching (8.0 vs. 1.0 mm; $Z = -2.371$; $p = 0.018$), but not for SBC2 compared to PLA ($p > 0.05$). Increases in the severity of nausea post-exercise was also observed following SBC3 compared to SBC2 ($Z = 2.366$; $p = 0.018$; **Figure 5A**), but not belching ($Z = 1.352$; $p = 0.176$; **Figure 5B**). There was no difference between aggregate GI upset between SBC2 and SBC3 at any time point (all $p > 0.05$).

DISCUSSION

This study is the first to explore the dose-response effects of $NaHCO_3$ ingestion when administered at a standardized time point on estimated energy system contribution and performance during intermittent cycling exercise. Both 0.2 and 0.3 g.kg^{-1} BM $NaHCO_3$ improved cycling TTE and estimated glycolytic contribution during HICT, therefore both doses can be employed as an ergogenic strategy. Only minimal dose-dependent differences in GI upset were observed, although the smaller dose mitigated severity of post-exercise nausea and belching. The key finding of this study therefore is that 0.2 g.kg^{-1} BM of $NaHCO_3$ can increase estimated glycolytic system contribution and be ergogenic for intermittent exercise performance.

Improvements in cycling TTE were observed for SBC2 and SBC3, with the moderate-to-large effect sizes reflective of previous findings employing a similar TTE protocol (26). The present study adds to previous work (17, 38), however, that ergogenic benefits can also be observed with a lower dose of $NaHCO_3$. Importantly, however, more participants improved over the SWC for SBC3 vs. SBC2, and a small effect size between treatments was observed in favor of SBC3 at the group level. This contradicts findings by McKenzie et al. (38) that displayed a 4 s difference in TTE for 0.15 and 0.3 g.kg^{-1} BM

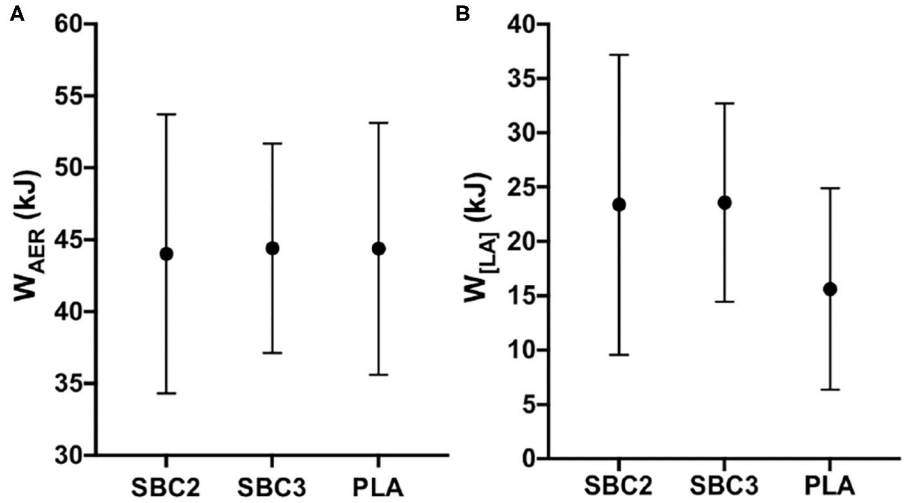

FIGURE 4 | (A,B) Mean ± SD for W_{AER} **(A)** and $W_{[LA]}$ **(B)** contribution during TTE cycling; SBC2, 0.2 g.kg^{-1} BM $NaHCO_3$; SBC3, 0.3 g.kg^{-1} BM $NaHCO_3$; PLA, sodium chloride (placebo).

$NaHCO_3$, and Gough et al. (12) where only a 0.1% variation in 4-km cycling time trial performance was present for 0.2 and 0.3 g.kg^{-1} BM doses. This discrepancy could be explained by differences in administration approach (standardized time point vs. time-to-peak), or the high-degree of inter-individual variation present in acid base balance following $NaHCO_3$ ingestion. Nonetheless, based on seven participants improving their performance following SBC3 vs. SBC2 (based on SWC), it is likely the athlete will secure the largest benefit from this higher dose. These dose-dependent differences in performance could also be attributed to the timing of exercise protocols. The cycling TTE protocol commenced ∼75 min after $NaHCO_3$ ingestion accounting for both the warm-up and HICT, however it is expected that [HCO_3–] will continue to rise until ∼80 min post-ingestion for SBC3, by which point [HCO_3–] will have started to decline for SBC2 in most individuals (12, 14). Nonetheless, athletes unable to pre-determine their time-to-peak HCO_3– can still employ either dosing strategy of the present study to obtain performance benefits during high-intensity cycling exercise.

Moderate, albeit non-significant, increases were observed for $W_{[LA]}$ during the HICT without altering energy demand or contribution from W_{AER}, which is in agreement to findings from recent studies (20, 21, 31). Despite not achieving statistical significance, these increases were considered substantial for both SBC2 (+15.8%) and SBC3 (+30.3%) when compared to PLA, with the relatively small absolute changes in $W_{[LA]}$ attributed to the controlled total mechanical work during the HICT (20). The most novel finding, however, was that there may be a dose-response effect of $NaHCO_3$ ingestion on changes in energy system contributions, with a small effect size present for $W_{[LA]}$ in favor of SBC3. Considering that enhanced HCO_3– buffering capacity is responsible for elevating glycolytic contribution, one explanation for these dose-dependent results could relate to the total amount of H^+ that can be neutralized. Assuming that total blood volume is ∼5 L and that [HCO_3–] was as small as ∼1.0 mmol.l^{-1} higher for SBC3 vs. SBC2, then the higher dose could have allowed the neutralization of an extra ∼5 mmoles of H^+ (based on the 1:1 stoichiometry of HCO_3– and H^+ reaction), in theory eliciting a greater up-regulation of glycolytic contribution (20). It is important to note, however, that as the current methodology only indirectly assesses glycolytic flux (i.e., from changes in [BLa^-]), these increases in $W_{[LA]}$ contribution may overestimate glycolytic activation, instead reflecting greater lactate efflux from working muscles (5). Nonetheless, previous research

TABLE 3 | The severest gastrointestinal (GI) symptoms for participants during each experimental trial.

Participant	SBC2	SBC3	PLA
1	**BUR (90.0)****	Vomiting (80.0)**	GF (39.0)
2	**BUR (19.0)**	GF (17.0)	GF (14.0)
3	**Belching (20.0)**	GF (18.0)	Belching (5.0)
4	**GF (24.0)**	**GF (24.0)**	Belching (23.0)
5	Nausea (31.0)	**AD (33.0)**	Nausea (23.0)
6	**GF (59.0)**	**GF (59.0)**	Belching (39.0)
7	**GF (12.0)**	GF (10.0)	Nil (0.0)
8	GF (39.0)	AD (31.0)	**GF (69.0)**
9	GF (10.0)	**Flatulence (49.0)**	Belching (21.0)
10	Flatulence (21.0)	**AD (71.0)**	AD (13.0)
11	**Nil (0.0)**	**Nil (0.0)**	**Nil (0.0)**
12	GF (17.0)	**GF (72.0)**	GF (66.0)

*Symptom scores (out of 100 mm) are displayed in parenthesis; SBC2, 0.2 g.kg^{-1} BM $NaHCO_3$; SBC3, 0.3 g.kg^{-1} BM $NaHCO_3$; PLA, sodium chloride (placebo); BUR, bowel urgency rating; GF, gut fullness; AD, abdominal discomfort; **Reported 5–10 min after laboratory visit; highest symptom severity for each participant highlighted in bold.*

has corroborated the findings of the present study following $NaHCO_3$ ingestion (23), therefore it seems plausible that both dosing strategies partially up-regulate glycolytic activation during high-intensity cycling.

The ingestion of $NaHCO_3$ resulted in mild-to-moderate GI symptoms, although both doses were well-tolerated, which agrees with previous research (14). Minimal dose-dependent differences were observed for GI upset, though the reduced post-exercise nausea and belching for SBC2 agrees with Gough et al. (12) where belching was exacerbated for the higher dose. The reduced severity of GI upset from this study could be attributed to the body mass of the participants in the present study (mean = 68 ± 6 kg) compared to those that have reported greater severity of GI upset in healthy males (15) and trained rugby players (10) (90 ± 6 and 95 ± 13 kg). Relative dosing protocols were derived during early laboratory studies to normalize post-exercise base deficit (39), and therefore fail to account for physiological differences such as body mass and the total absolute $NaHCO_3$ dose. Athletes with high body mass administer a greater absolute $NaHCO_3$ dose despite minimal differences in gut absorption rates, particularly for the first 60 min post-ingestion (14), which most likely exacerbates GI upset. There might be an upper threshold for absolute $NaHCO_3$ doses, with doses above this exacerbating GI upset. At present, 0.2 $g.kg^{-1}$ BM $NaHCO_3$ is a suitable strategy for mitigating GI upset; however, future research could examine the effect of absolute dosage on symptom severity and exercise performance.

There are methodological limitations in the present study that future research should address. Firstly, the single-blind design of this study is a limitation that is important to note. Important methodological choices were adopted, however, to mitigate any potential impact of this design. This included the standardized verbal encouragement during exercise, and the use of a supplement belief questionnaire, as per previous research (12). The findings from the latter methodological decision suggested that the supplement was blinded from the participants and therefore the single-blind design has no impact on the efficacy of $NaHCO_3$ ingestion. Moreover, our inability to quantify changes in absolute demand and contribution from the ATP-PCr energetic system is a limitation. This was due to the relatively short recovery period (90 s) between each bout of the HICT that did not allow a clear EPOC curve to form and therefore, it was decided that the ATP-PCr energy contribution calculations should be excluded from our analysis. Lastly, it was not possible to measure changes in [HCO_3–] following $NaHCO_3$ ingestion in the present study. Evidence suggests, however, that the HCO_3– response is similar for 0.2 and 0.3 $g.kg^{-1}$ BM $NaHCO_3$ doses within ∼60 min, therefore participants were likely at a similar level of alkalosis irrespective of dose (12, 14). This timing of $NaHCO_3$ ingestion employed in this study was selected to assess of the potential ergogenic effects for athletes unable to adopt an individualized time-to-peak HCO_3– approach, or access a blood gas analyser. Based on the observed ergogenic benefits for both doses vs. PLA, it should further enhance the practical application of $NaHCO_3$ supplementation to the athlete with limited funding.

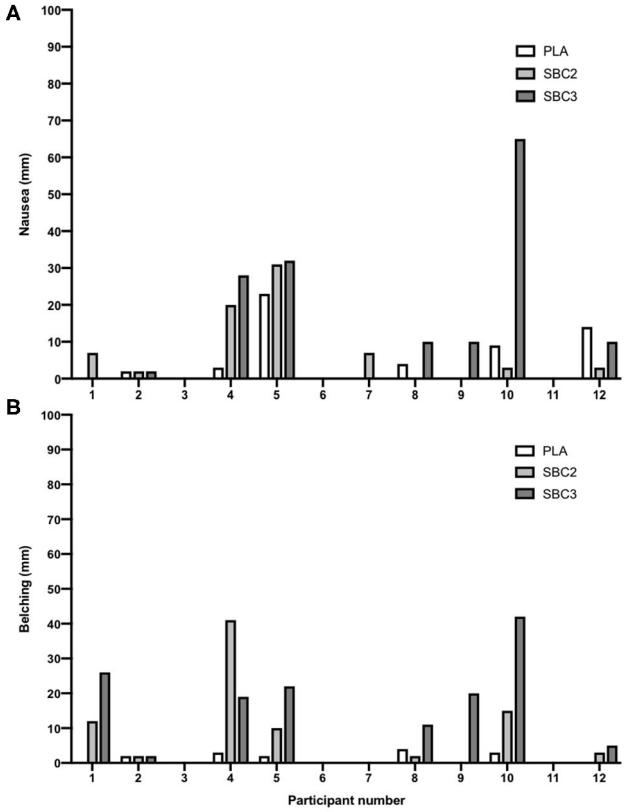

FIGURE 5 | (A,B) Inter-individual variations in post-exercise nausea and belching; self-reported symptoms via visual analog scales (out of 100 mm); SBC2, 0.2 $g.kg^{-1}$ BM $NaHCO_3$; SBC3, 0.3 $g.kg^{-1}$ BM $NaHCO_3$; PLA, sodium chloride (placebo).

CONCLUSION

Ingestion of 0.2 and 0.3 $g.kg^{-1}$ BM elevated glycolytic contribution to high intensity exercise and are ergogenic strategies to improve exercise performance. It is likely that athletes will gain increased benefit from SBC3, despite the occurrence of higher GI upset. Nonetheless, some athletes may still opt for the lower dose if this displays greater tolerability, whilst still securing an ergogenic benefit. The present study also shows that the contemporary time to peak alkalosis strategy might not be required when ingested 60 min prior to exercise, however direct comparisons between these two methods of ingestion are required.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Essex. The patients/participants

provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KR, WG, and LG designed the study. WG completed the data collection, whilst WG and LG completed the majority of the manuscript, MF, SS, and KR also contributed. All authors reviewed the paper and provided feedback. LG and WG completed the preparation of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Impact of Individualizing Sodium Bicarbonate Supplementation Strategies on World-Class Rowing Performance

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Contemporary meta-analyses have generally demonstrated a positive effect of sodium bicarbonate ($NaHCO_3$) supplementation on exercise performance. However, despite these claims, there is limited data on contrasting individualized and standardized *timing* of $NaHCO_3$ ingestion prior to exercise to further enhance performance outcomes.

Purpose: To determine whether $NaHCO_3$ ingestion timing impacts 2,000-m rowing time-trial (TT) performance in elite-level rowers (Senior National team including Olympic/World Championships level) adhering to their own individualized pre-race strategies (e.g. nutrition, warm-up, etc.).

Methods: Twenty three ($n = 23$) rowers across two research centers (using the exact same methods/protocols) completed three trials: $NaHCO_3$ loading profile at rest to determine the individual's time-to-peak bicarbonate concentration [HCO_3^-], followed by two randomized 0.3 g·kgBM^{-1} $NaHCO_3$ supplementation experimental trials conducted at different time points [consensus timing (CON): TT performed 60 min post-$NaHCO_3$ ingestion; and individualized peak (IP): TT performed at the rower's individual peak [HCO_3^-] determined from the profiling trial post-$NaHCO_3$ ingestion].

Results: There was a significant mean difference of +2.9 [± 0.4 mmol·L^{-1} HCO_3^- for IP vs. CON (95% CI 2.0 to 3.8 mmol·L^{-1}); $p = 0.02$; $d = 1.08$] at pre warm-up, but not immediately prior to the TT (post warm-up). Performance times were significantly different between IP (367.0 ± 10.5 s) vs. CON (369.0 ± 10.3 s); $p = 0.007$; $d = 0.15$).

Conclusions: The present study demonstrated a small but significant performance effect of an individualized $NaHCO_3$ ingestion strategy. Similarities after warm-up between pre-TT $sHCO_3^-$ values (CON ∼ + 5.5 mmol·L^{-1}; IP ∼ + 6 mmol·L^{-1}), however, would suggest this effect was not a result of any meaningful differences in blood alkalinity.

Keywords: sodium bicarbonate ingestion, individualized nutrition, time trial performance, elite athletes, performance

INTRODUCTION

The most recent 2018 International Olympic Committee (IOC) sports nutrition consensus statement recommendations have suggested that sodium bicarbonate ($NaHCO_3$) is one of five dietary supplements that has generally been shown to improve performance in the elite athlete (1). Indeed, there are a number of contemporary meta-analyses demonstrating the potential performance efficacy of supplementing with $NaHCO_3$ as compared to placebo in sports where perturbations in cellular buffering capacity influences performance (typically in events of 1–10 min) (2–5). Current consensus statement ingestion recommendations are to consume between 0.2 and 0.4 g·kg body mass $(BM)^{-1}$ with a small, carbohydrate (CHO) dense meal ($\sim$1.5 g·kgBM^{-1} CHO) $\sim$60 to 150 min prior to exercise (1). However, as contemporary papers have also highlighted, these recommendations serve only as a starting point when considering $NaHCO_3$ supplementation for the individual athlete (6–8). A recent review by Heibel et al. has addressed several practical issues associated with traditional $NaHCO_3$ supplementation approaches, identifying ingestion timing as potentially critical to maximizing the effectiveness of this supplement (6). Given the existing scientific support for the use of $NaHCO_3$ (2–5), as well as the high prevalence of use [e.g., rowing (9–12)], understanding the influence of ingestion timing under ecologically valid conditions may further improve the effectiveness of this supplement. To date no study has collectively investigated the relationship between $NaHCO_3$ timing, buffering capacity, gastro-intestinal (GI) distress, and pre-race nutritional recommendations coupled to performance outcomes in world-class athletes.

The premise for individualizing $NaHCO_3$ supplementation has both historical (8, 13) and more recent scientific support (14–16). Several contemporary publications have consistently demonstrated the high degree of inter- and intra-individual variability often observed during $NaHCO_3$ studies, despite dosing by body mass (kg) and standardizing pre-supplementation nutrition and fluid intake (7, 8, 17). Furthermore, a relatively recent study has profiled the large individual variations in blood bicarbonate (HCO_3^-) in response to ingesting 0.1, 0.2, and 0.3 g·kg^{-1} $NaHCO_3$ (7). These authors also underscored the large variation in time-to-peak blood buffering capacity (e.g., highest recorded [HCO_3^-]) between the participants (range from 30 to 180 min) (7), despite a per kilogram body mass dosing regimen and 24-h dietary replication protocols. Subsequently, these data have been followed by a series of studies assessing the intra-individual reproducibility of blood buffering profiles (14, 16, 18) and exercise performance under varying doses of $NaHCO_3$ (14, 16). Collectively, these studies provide preliminary evidence suggesting that adjusting the start of a competitive effort to commensurate with an individual's peak blood buffering response at rest may result in better outcomes in terms of GI distress and exercise performance (14, 16, 19).

Although promising, the importance of timing performance trials to coincide with an individual's peak blood buffering capacity has not yet been investigated in world-class level athletes. For example, neither of the aforementioned studies investigating reproducibility and performance (14, 16) introduced $NaHCO_3$ as it would be to a competitive athlete (e.g., in a fed rather than fasted state, in capsule rather than liquid-based solution and under the pressure of competitive situations). Moreover, neither of these studies have investigated the effect of performance timing. Miller et al. compared an individualized $NaHCO_3$ ingestion protocol to a placebo and control trial in recreationally active individuals (16), whilst Gough et al. investigated the reproducibility of cycling time-trial performance under varying (but all individualized) dosages of $NaHCO_3$ (14). We therefore designed this proof-of-concept study to specifically address the question of whether or not ingestion *timing* influences time-trial performance [2,000-m rowing time-trial (TT)] in elite-level rowers adhering to their own individualized pre-race strategies (e.g., nutrition, warm-up, etc.). Incorporating the 2,000-m rowing TT under these conditions of high ecological validity also provided the opportunity to further explore the complex, inter-related issues surrounding $NaHCO_3$ ingestion, GI-distress, and acid-base balance; all concerns raised by previous investigations in this field (9, 11, 20). We hypothesized that 2,000-m rowing times would be improved when the TT commenced at an individual's peak blood buffering capacity as compared to a start time corresponding with the minimum IOC recommendations (60 min) (1).

MATERIALS AND METHODS

A multi-center approach was utilized to maximize the number of international competitive rowers involved. Twenty three (n = 23) elite rowers [lightweight rowers (n = 4), body weight 73.6 ± 2.1 kg; open-class rowers (n = 19), 93.6 ± 5.8 kg; mean (M) ± standard deviation (SD)] were recruited across two research centers [Canadian Sport Institute Pacific (CSIP; Canada) and the New South Wales Institute of Sport (NSWIS; Australia)] with identical methods and protocols conducted at both institutes. Participants were male competitive rowers able to complete a 2,000-m ergometer TT at or below 6 min 20 s [Participant 2,000-m ergometer TT personal bests (PB) ranged from 5 min 39 s (open-weight) to 6 min 14 s (light-weight) and included 13 Olympic/World-Champs team members as well as one rowing ergometer world record holder]. Fifteen of the 23 athletes all had previous experience ingesting $NaHCO_3$ in various contexts, while the remaining eight (U23) were only aware of the potential benefits. All participants were informed verbally and in writing as to the nature and risks associated with the study, submitted to health screening and gave their written informed consent. All procedures in this study were approved by the respective ethics committees (Australian Institute of Sport Ethics Committee; approval code 20171205 and the University of Victoria Human Research Ethics Board; approval code 18-045) and were conducted in accordance with the Declaration of Helsinki.

Pilot Study

Prior to the investigation, an ingestion protocol was implemented (see section Experimental Trials) to assess (a) the reliability of the ABL 80 Flex (Radiometer, Copenhagen, DK); (b) week-to-week

repeatability of pH and standard (s) HCO_3^- after a standardized ingestion of 0.3 g·kg^{-1} $NaHCO_3$ in both study locations (inter and intra-reliability); and (c) to define objective parameters for identifying an athlete's individual time-to-peak blood buffering capacity [highest recorded ($sHCO_3^-$)] after $NaHCO_3$ ingestion [Note: $sHCO_3^-$ is only used in reference to the data collected in this study, whereas the abbreviation HCO_3^- is used in all other instances (e.g., blood bicarbonate or other published papers unless otherwise noted)]. A total of $n = 8$ volunteers (four in each institute) completed two passive ingestion trials, conducted at the same time of day, each separated by 1-week and after the same dietary replication (see section Dietary Controls).

To facilitate arterialization, each seated participant's hand was warmed (either by heated pad or warm water) 10 min prior to obtaining a baseline sample and throughout the entire profiling session. For baseline and all subsequent measures (every 10 min for a total of 150 min starting 30 min after the final $NaHCO_3$ pill was ingested), whole blood was collected in duplicate from the finger-tip into a heparinized 120 µl blood gas capillary tube and immediately analyzed for acid-base status (pH and $sHCO_3^-$; ABL 80 Flex). After the baseline sample, participants consumed gelatin capsules providing a total of 0.3 g·kgBM^{-1} of $NaHCO_3$ (1,000 mg $NaHCO_3$ per capsule over a 30 min period (e.g., 1/3 ingested at 0, 15 and 30 min) with a standardized snack and 10 ml·kgBM^{-1} fluid. The snack was designed to replicate typical pre-competition practice (with maltodextrin added to fluids where necessary to standardize carbohydrate (CHO) intake at 1.5 g·kgBM^{-1}) and minimize potential GI discomfort (20). All $NaHCO_3$ capsules were third-party batch tested by LGC Limited (Lexington KY, US; certification number 22,948) for prohibited substance contamination against the World Anti-Doping Association List.

General Procedures

A study overview is provided in **Figure 1**. Each participant reported to the temperature controlled Exercise Physiology Laboratory's at CSIP and NSWIS on three separate occasions (loading profile followed by two randomized and athlete single-blinded deception (as outlined below) experimental trials) at the same time of day for each trial and separated by >5 and <14 days. Familiarization with this world-class cohort was established over a series of regular, 2,000-m TT efforts on the ergometer recorded during normal training and over a 12 month period prior to the study (participants had been competitive rowing training for $\sim 9 \pm 3$ years with an average of $3 \times$ 2,000-m TT tests/year). To implement high ecological validity, participants replicated their typical 24 h pre competition nutrition practices (macronutrient composition, volume, and timing) prior to all three trials (see section Dietary Controls).

Individualized $NaHCO_3$ Loading Profile

Participant preparation and ingestion procedures were conducted as presented previously (see section Pilot Study). At 30 min post snack, capillary sampling (in duplicate) re-commenced, where samples were obtained and analyzed every 10 min until a plateau in $sHCO_3^-$ was identified. The plateau was determined to have occurred when the change in three consecutive measurements were smaller than the ABL 80 technical error of measurement (TEM) (0.4 mmol·L^{-1}; see section Results: Pilot Study). Sampling continued until two consecutive measurements indicated a decline in [$sHCO_3^-$] greater than the TEM associated with identifying peak blood buffering capacity (0.6 mmol·L^{-1}; see section Results: Pilot Study). Time-to-peak (min) for the IP trial was thereafter defined as the final measurement time point prior to this decline. Toilet breaks and walks around the room were considered an acceptable level of activity and GI discomfort was documented upon arrival to the laboratory and throughout this sampling period (see section Gastrointestinal Profile). Body weight was measured upon arrival to the laboratory, before and after all toilet visits and finally at the end of sampling (Avery Berkel, Model HL120, Smethwick, UK). Total fluid volume over the loading period was also recorded, along with urine specific gravity (USG; Atago PAL-10S, Tokyo, JP) at the start of the trial to ensure similar biological states between trials. From the blood acid-base data collected over the 180 min time period, peak [$sHCO_3^-$] and time-to-peak after $NaHCO_3$ ingestion was identified for the purpose of timing the start of the two subsequent experimental trials.

Experimental Trials

After completing the individualized $NaHCO_3$ loading profile, two experimental exercise performance trials were randomly administered under the following timing conditions:

- Minimum IOC consensus timing recommendation (CON): 2,000-m rowing TT performed 60 min post ingestion of 0.3 g·kgBM^{-1} $NaHCO_3$.
- Individualized Timing Peak (IP): 2,000-m rowing TT performed at the participant's individual peak [$sHCO_3^-$] (determined from the profiling) after ingesting 0.3 g·kgBM^{-1} $NaHCO_3$.

As the primary aim of this study was to test whether or not it is critical to commence exercise performance trials at an individual's peak blood buffering capacity, the CON trial needed to start within a time-frame that was sufficiently supported by evidence (2–5). Therefore, when considering the available evidence suggesting individualized $NaHCO_3$ supplementation might extend out the peak buffering capacity timeframe for most athletes (8, 14, 16, 18), we assert that investigating the earliest time supported by IOC guidelines (60 min) (1) compared to individual athlete time-to-peak had the potential to provide the best proof-of-concept scenario to test this effect (particularly given the logistical restraints of working with this elite population).

For the two experimental trials, participants were asked to replicate their training for 48 h before each TT and to also replicate their 24 h dietary intake (see section Dietary Controls) prior to the initial $NaHCO_3$ loading profile trial. For both experimental conditions, a dose of 0.3 g·kgBM^{-1} of $NaHCO_3$ was administered orally in gelatin capsules following the same ingestion protocol identified previously (see section Individualized $NaHCO_3$ Loading Profile). Capillary blood was sampled for acid-base balance [(pH and $sHCO_3^-$), see section Pilot Study] and blood lactate (BLa) (The Edge; Woodley

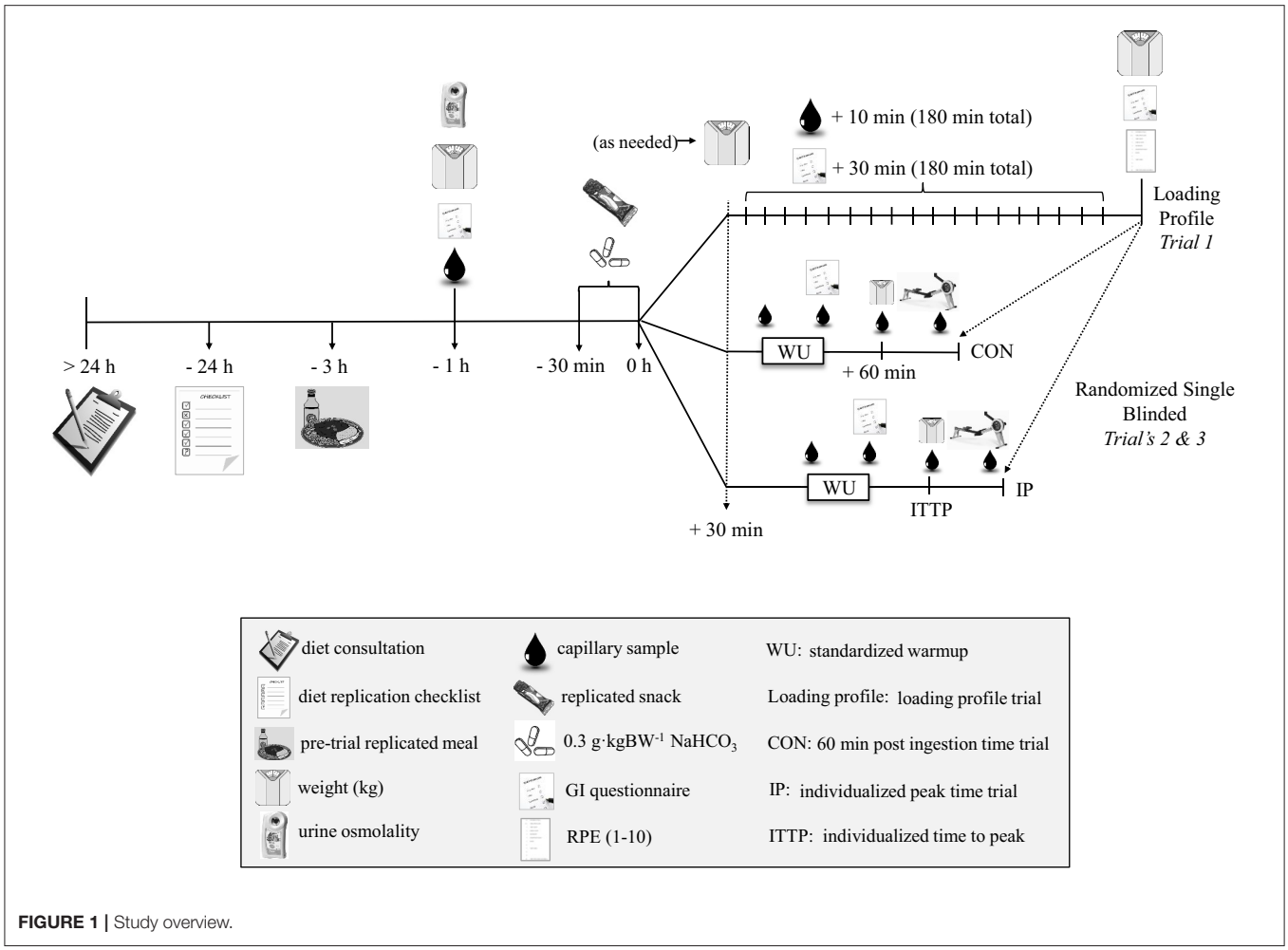

FIGURE 1 | Study overview.

Equipment Company, Bolton, UK) pre-ingestion (Base) (with the exception of BLa), pre-warm-up (Pre-WU), 1 min post lactate push (Post-Push), pre time trial (Pre-TT) and 1 min post completion of the TT (Post-TT). Rating of Perceived Exertion (1–10 Borg scale; RPE) was obtained at the end of each TT. Gastrointestinal symptoms were again documented throughout each experimental trial. Body weight was assessed pre-capsule ingestion and again pre-TT to monitor any fluid changes induced by $NaHCO_3$.

The 2,000-m rowing TT was performed on Concept2 rowing ergometers with the display screen blinded to give only distance completed feedback (Model D Concept2, Inc., Morrisville, Vermont, US) after participants replicated their usual, pre-competition warm-up in the laboratory. Individual pre-TT warmup (reviewed by a sports physiologist) occurred under the following guidelines: category 6 (C6; lowest intensity) "erging" with a lactate priming effort of 1 min at 2,000-m race pace completed 20 min before the start of the TT [shown to improve high-intensity TT's (21)]. Participants were able to stay warm throughout the post lactate push period with C6 or lower intensity erging, interspersed with periodic power strokes (PS). No more than 3 to 5 PS/set, with no more than 2 to 3 sets and a minimum of 5 min between sets, was permitted. For the 2,000-m TT, participants were asked to perform the 2,000-m row on their own with the only feedback being distance completed. No verbal encouragement was provided.

In this world-class cohort, all of the rowers had prior knowledge of, or experience using $NaHCO_3$ and were aware of the contemporary approaches (e.g., individualized timing protocols) being trialed in various sports. To properly mitigate any performance expectancy related to individualized supplement timing, a quadruple cross-over design would have been required. Four, 2,000-m TT's was not possible, therefore single-blinded deception around whether or not the athletes received $NaHCO_3$ was used to mask the potential belief that an individualized timing protocol might benefit performance over a standard timing (22). Therefore, although participants received $NaHCO_3$ prior to both 2,000-m rowing TT sessions (CON and IP), they were informed in advance of the study that they would be randomly assigned to either placebo or $NaHCO_3$ for either the CON or IP trials. Participants were informed that the placebo would contain calcium carbonate, and would be designed to look, taste and produce side effects similar to the $NaHCO_3$ capsules without the possible performance enhancing effects.

TABLE 1 | Mean ± SD macronutrient profile [carbohydrate (CHO), protein, fat], fiber and total energy (TE) during the 24 h prior to the trials and the Pre-TT snack for both IT and CON.

	CHO (g)	Protein (g)	Fat (g)	Fiber (g)	TE (kcals)
24 h profile (not including snack)	793 ± 402	263 ± 130	222 ± 113	60 ± 28	6,125 ± 2,862
Pre-TT snack	140 ± 16	20 ± 27	10 ± 10	7 ± 2	682 ± 121

After each trial participants completed a deception questionnaire to determine the success of the blinding (63% believed the CON trial was the placebo supplement, when in fact, participants received $NaHCO_3$ 100% of the time suggesting this approach was successful).

Dietary Controls

Prior to the individualized $NaHCO_3$ loading profiling each athlete reviewed and recorded their typical 24-h pre-competition diet with an experienced Sports Dietitian to standardize dietary intake while replicating their usual pre-competition (pre-race or pre-TT) nutrition practice. Timings of meals and snacks were optimized around training, with the last substantial "pre-race" meal standardized to be completed 3 h prior to the ingestion of the $NaHCO_3$ load. Participants were provided with a detailed diet checklist based on their recall and asked to replicate before each trial [verbally confirmed prior to each trial (**Table 1**)].

Gastrointestinal Profile

Participant GI-symptoms were documented before and immediately following the sampling period of the $NaHCO_3$ loading profile assessment, as well as pre- and post-TT in the experimental trials, using a 100 mm Visual Analog Scale (VAS) for eight different GI-symptoms (nausea, flatulence, bloating, belching, stomach-ache, bowel urgency, diarrhea and vomiting) as adapted from Pfeiffer et al. (23).

Statistical Analysis

For the pilot study intraclass correlation analysis (two-way mixed effects model ICCs with 95% Confidence Intervals) in conjunction with the typical error of measurement (TEM) statistic was applied to assess the reliability of the ABL 80 Flex and week-to-week repeatability (two weeks where duplicate samples were averaged for each week) of pH and $sHCO_3^-$ (24). TEM of peak $sHCO_3^-$ values were also determined using the change scores from baseline to peak value for each sampling period during the 2 weeks. Finally, time-to-peak $sHCO_3^-$ was recorded and used in conjunction with the TEM of peak $sHCO_3^-$ to provide an objective framework to determine the ingestion timing sequence for the IP trial.

For the experimental trials, descriptive data are presented as mean ± SD with all statistical analyses being completed using IBM SPSS Statistics version 25 (SPSS Inc., Chicago, US). Changes in blood acid-base, lactate profiles and body weight throughout the experimental trials were analyzed using a two-way ANOVA for repeated measures. In the event of a significant F ratio, *post hoc* comparisons were made using a Bonferroni correction.

TABLE 2 | Reliability data [Intraclass Correlation Coefficients (ICC), Confidence Intervals (CI), and Typical Error of the Measurement (TEM)] collected during the *Pilot Study* for pH and $sHCO_3^-$ measures obtained from the ABL 80 Flex (Radiometer, Copenhagen, DK).

Within-sample reliability	ICC (95% CI)	TEM (95% CI)
pH (au)	0.76 (95% CI 0.66 to 0.84)	0.02 (95% CI 0.02 to 0.04)
$sHCO_3^-$ ($mmol{\cdot}L^{-1}$)	0.99 (95% CI 0.98 to 0.99)	0.4 (95% CI 0.3 to 0.5)
Week-to-week variability	**ICC (95% CI)**	**TEM (95% CI)**
pH (au)	0.30 (95% CI 0.04 to 0.59)	0.04 (95% CI 0.03 to 0.05)
$sHCO_3^-$ ($mmol{\cdot}L^{-1}$)	0.77 (95% CI 0.59 to 0.89)	1.5 (95% CI 1.2 to 1.9)

Mean differences and standard error (SE) between conditions as well as 95% confidence intervals (CI) were calculated when significant changes occurred over time, or when differences between conditions were observed. 2 km TT performance times (s), subjective exertion (RPE) and pre-trial USG recorded during the two experimental trials were compared using paired *t*-tests, with significant differences further evaluated for effect size using the Cohen's *d* statistic (0.2, 0.5, and 0.8 corresponding to small, medium and large effects, respectfully). Two-tailed statistical significance was accepted at $p < 0.05$.

RESULTS

Pilot Study

Within-sample and week-to-week variability of the ABL 80 Flex for both pH and $sHCO_3^-$ is provided in **Table 2** and **Figure 2**. TEM of peak $sHCO_3^-$ values were 0.6 $mmol{\cdot}L^{-1}$ (95% CI 0.4 to 1.6 $mmol{\cdot}L^{-1}$), with an average variation of 14 ± 12 min (range 0 to 30 min) in time-to peak $sHCO_3^-$ observations.

Experimental Trials

Blood Parameters

Significant time effects were evident across the trial period for pH ($F = 477.1$; $p < 0.001$; $\eta^2 = 0.97$) and $sHCO_3^-$ ($F = 659.3$; $p < 0.001$; $\eta^2 = 0.98$) and consistent with induced states of metabolic alkalosis at Pre-WU and Pre-TT for both CON and IP (**Figure 3**). Significant interaction effects (pH: $F = 4.5$; $p < 0.01$; $\eta^2 = 0.22$; HCO_3^-: $F = 21.0$; $p < 0.001$; $\eta^2 = 0.57$) and *post hoc* comparisons revealed differences between CON and IP at Pre-WU (mean difference of 0.03 ± 0.01 au (95% CI 0.02 to 0.04 au); $p < 0.001$) and Pre-TT (mean difference of 0.02 ± 0.01 au (95% CI 0.003 to 0.03 au); $p = 0.02$) for pH, but only at Pre-WU for $sHCO_3^-$ (mean difference of 2.9 ± 0.4 $mmol{\cdot}L^{-1}$ (95% CI 2.0 to 3.8 $mmol{\cdot}L^{-1}$); $p = 0.02$; **Figures 3A,B**). Only a main effect of time was evident in BLa ($F = 44.4$; $p < 0.001$; $\eta^2 = 0.75$), with Pre-WU lower than Post-Push (mean difference of 3.6 ± 0.5 $mmol{\cdot}L^{-1}$ (95% CI 5.0 to 2.1 $mmol{\cdot}L^{-1}$); $p < 0.001$) and Pre-TT lower than Post-TT (mean difference of 15.4 ± 2.2 $mmol{\cdot}L^{-1}$ (95% CI 22.2 to 8.6

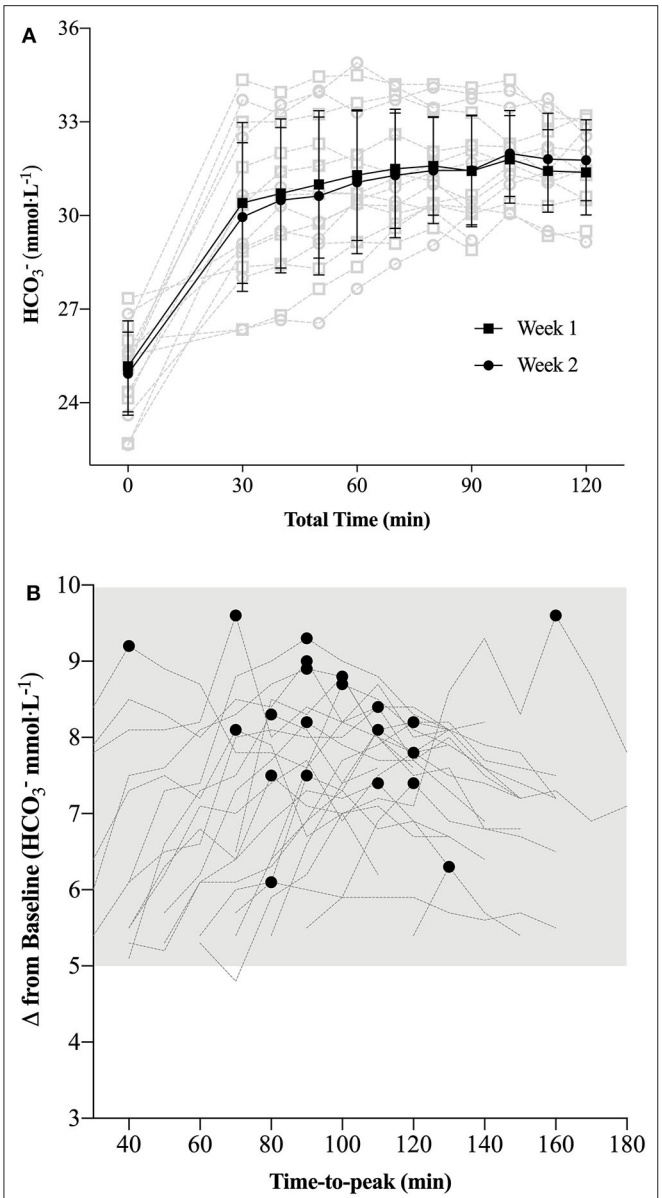

FIGURE 2 | (A) Week-to-week standard bicarbonate concentrations ([$sHCO_3^-$]) (mean ± SD: black; individual data: light gray) observed during the pilot study [$n = 8$; **(A)**]; **(B)**: Individual ($n = 23$) participant measurements of peak and corresponding time-to-peak as observed during the *Individualized $NaHCO_3$ Loading Profile* trial. Each individual's time-to-peak was subsequently used to demarcate the time frame prior to commencing the time trial (TT) in the *Individualized Peak* (IP) trial.

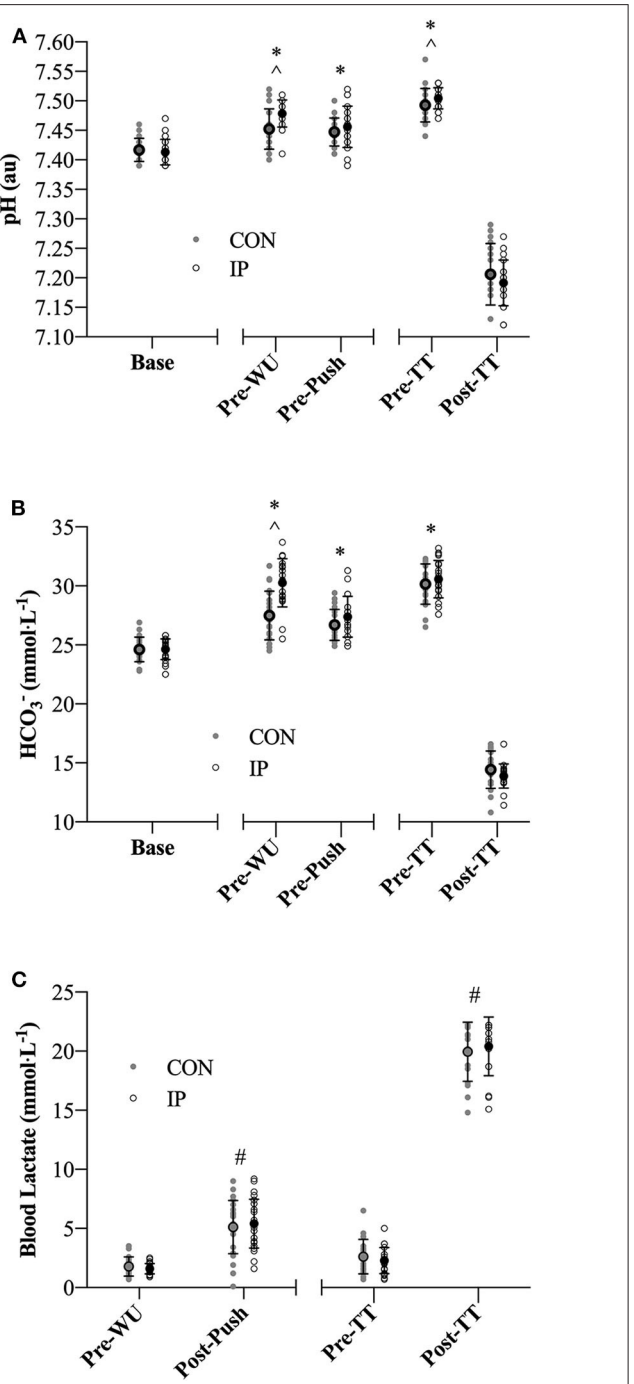

FIGURE 3 | (A–C) Blood acid-base and lactate (BLa) measurements obtained at baseline (Base; with the exception of BLa), pre-warm-up (Pre-WU), 1 min post lactate push (Post-Push), pre time trial (Pre-TT) and 1 min post completion of the TT (Post-TT). Individual and mean ± SD data are presented for both the consensus standard (CON; gray dots) and individualized peak (IP; open black circles) trials.

mmol·L^{-1}); $p < 0.001$) being significantly higher than Pre-WU and Pre-TT (**Figure 3C**).

2,000-m TT Performance

Performance times were significantly different between CON (369.0 ± 10.3 s) and IP (367.0 ± 10.5 s) [mean difference 1.5 ± 2.4 s (95% CI 0.5 to 2.6 s); $p = 0.007$; $d = 0.15$; **Figure 4**]. Of the 23 rowers, 18 improved their times in the IP trial with 11

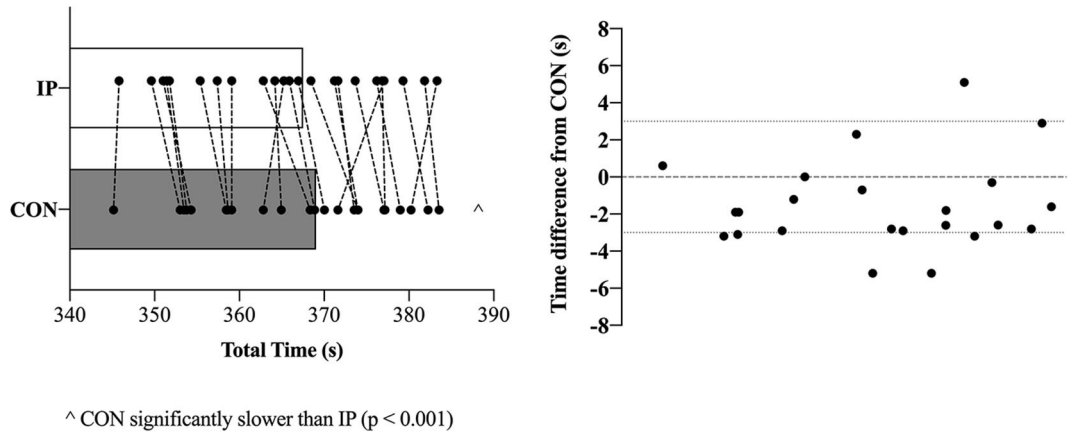

FIGURE 4 | Two thousand-metres rowing time trial (TT) performance times in seconds (individual and mean ± SD data) for both the consensus standard (CON; gray bar) and individualized peak (IP; open black border) trials (left). Individual time differences (+ or – in (s) from CON) are presented in the figure on the right.

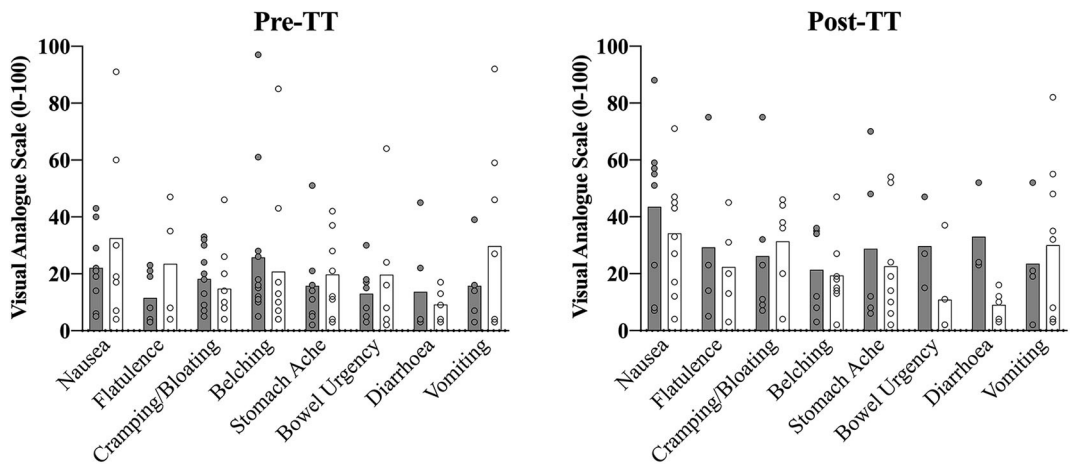

FIGURE 5 | Summed descriptive (individual and mean ± SD) symptom data collated from the GI questionnaire (0–100 point visual analog scale) prior to the start of the 2,000-m TT (Pre-TT) and upon completion (Post-TT) for both the consensus standard (CON; gray bar) and individualized peak (IP; open black border) trials.

participants at or above a 3 s improvement (**Figure 4**). There were no differences in ratings of perceived exertion scores between CON (8.9 ± 1.1) and IP (9.2 ± 0.9) after completing the TT ($p = 0.42$).

USG, Body Weight and GI Symptoms

USG was not different prior to the two experimental trials (CON: 1.013 ± 0.008; IP: 1.015 ± 0.008; $p = 0.20$). Significant time effects were evident across the trial period for BM ($F = 5.6$; $p < 0.01$; $\eta^2 = 0.24$), with a mean increase in weight of 0.35 ± 0.13 kg (95% CI 0.10 to 0.68 kg; $p = 0.04$) from Base to Pre-WU. Post-TT weights had returned to Base levels and were not different (Base: 89.77 ± 2.3 kg; Post-TT: 89.84 ± 2.2 kg; $p = 1.0$). No differences were evident between conditions ($p = 0.33$). For descriptive purposes only, GI symptoms are presented (mean ± SD) for each symptom at Pre-TT and Post-TT during the CON and IP trials (**Figure 5**).

DISCUSSION

A growing body of evidence suggests a number of factors may affect the efficacy of the supplement $NaHCO_3$ as a strategy to mitigate fatigue in the context of exercise performance (7, 8, 14, 16–18). These factors are primarily related to the complex interplay between ingestion strategies as they relate to changes in peak blood buffering capacity (e.g., dose-response), GI distress and ingestion timing. The aim of the present study was to specifically address the issue of ingestion *timing* by examining whether adjusting start times to coincide with an individual's peak blood buffering capacity after $NaHCO_3$ supplementation would influence 2,000-m rowing TT performance. Our approach of individualizing the time-to-peak was successful as Pre-WU [$sHCO_3^-$] was nearly 3 mmol·L^{-1} greater ($p = 0.02$) for IP than CON (**Figure 3B**), although this difference diminished in the experimental trials after the addition of the warm-up

(Pre-TT $sHCO_3^-$ values: CON $\sim$ + 5.5 mmol·L^{-1}; IP $\sim$ + 6 mmol·L^{-1}). The present study also demonstrated a small but significant performance effect of an individualized $NaHCO_3$ ingestion strategy [IP (367.0 ± 10.5 s) vs. CON (369.0 ± 10.3 s); $p = 0.007$; $d = 0.15$]. Moreover, 18 of the 23 participants improved their times in the IP trial, with 11 participants at or above a 3 s improvement (**Figure 4**). Given the caliber of the athletes in this study, these findings provide preliminary support for individualizing ingestion timing strategies.

A number of recent independent investigations have clearly demonstrated the high degree of inter-individual variability associated with time-to-peak buffering capacity after $NaHCO_3$ ingestion, reporting peak HCO_3^- concentrations ranging between 10 and 150 min post ingestion after a similar dose of 0.3 g·kgBM^{-1} (7, 18, 25). Indeed, the inter-individual variability has also been cited as a possible contributing factor toward inconsistencies of erogenicity observed in some studies (8, 17), and highlighted in contemporary reviews as an area for further study (6, 26). With specific reference to rowing, it is plausible that this inter-individual variability may have influenced the performance outcomes of many of the studies observing minimal to no effect of $NaHCO_3$ supplementation (9–12, 27). Although we observed less than a two second ($\sim$ 0.5%) difference in performance times (**Figure 3**), in practical terms this would equate to greater than a boat's length in competition. Even in this world-class cohort where we consistently observe 2,000-m CV's of 0.5 to 1.4% (28), the small but positive effect (d = 0.15) may be worth considering when developing a supplementation strategy for the elite competitor, particularly in light of the prevalence of small effects identified in this cohort (5). Though our findings tentatively support the concept of individualizing timing strategies with this supplement, further research is required to determine whether performance outcomes after individualization is due to differences in absolute changes blood [HCO_3^-] or some other mechanism (14, 16, 18).

Although speculative, as we only investigated the effect of timing, the results of the present study may also suggest that the absolute change in blood buffering capacity (**Figures 3A,B**), rather than timing exercise commencement to coincide at an individual's peak blood HCO_3^- concentration, may be more relevant when considering $NaHCO_3$ as a supplement. As demonstrated in a recent review (6), the ergogenic potential of consuming 0.3 g·kgBM^{-1} $NaHCO_3$ appears to improve substantially when the concentration of blood HCO_3^- is >5 to 6 mmol·L^{-1} above typical values (found in 17 of 19 studies reviewed). In the present study both CON ($\sim$ + 5.5 mmol·L^{-1}) and IP ($\sim$ + 6 mmol·L^{-1}) $sHCO_3^-$ concentrations were elevated above 5 mmol·L^{-1} prior to the start of each of the respective time trials. Furthermore, during the *NaHCO$_3$ Loading Profile* the time duration post-supplementation that participants were > 5 mmol·L^{-1} was $\sim$ 100 ± 20 min (range 40 to 160 min; **Figure 2B**), respectively; suggesting the potential for a buffering performance window. Though the theoretical premise of a minimal buffering threshold (e.g., 5 to 6 mmol·L^{-1}) or buffering window has merit, further research is required to directly test these hypotheses within the context of "real-world" performance parameters.

In support of the previous statement, the similar elevation in pre-TT [$sHCO_3^-$] observed in both performance trials (**Figure 3B**) also clearly demonstrates the effect of a typical high-intensity warm-up on blood acid-base kinetics, as only one participant achieved peak blood buffering capacity at the 60 min post-ingestion time point during the passive profiling trial (**Figure 2B**). This finding has practical significance, as the warm-up in this study was constructed by each individual athlete (within prescribed guidelines) and mimicked their own "pre-race" warm-up strategy. Presumably, either the proportion of high-intensity efforts or warm-up length facilitated an increased rate of HCO_3^- appearance in the blood as compared to a purely passive ingestion environment, essentially equating the buffering capacity between the two conditions. Irrespective of the similar pre-TT [$sHCO_3^-$], we ultimately cannot dismiss the performance improvement observed in the IP trial (**Figure 4**). Given the similar GI responses (**Figure 5**) and blood buffering concentrations between trials, we cannot speculate as to causation in this regard. In practice, however, it may be unnecessary to undertake the costly and time-consuming exercise of identifying an individual athlete's peak blood buffering capacity, when measured baseline and a "one-off" measure just prior to exercise will ensure HCO_3^- concentrations are significantly elevated (e.g., > 5 mmol·L^{-1}) post supplementation.

Presently, there are no universally accepted methods for determining peak blood buffering capacity after $NaHCO_3$ ingestion. Miller et al. used a single visit where peak [HCO_3^-] and pH values were visually determined during 160 min of sampling (16). Similar to our pilot study results (**Figure 2A**), after demonstrating a high degree of reproducibility in time-to-peak [HCO_3^-] (18), Gough et al. also used a single visit to determine time-to-peak HCO_3^- over a 180 min time frame post 0.2 and 0.3 g·kgBM^{-1} $NaHCO_3$ ingestion (14). Commendably, these authors reported individual participant data to complement the mean ± SD absolute HCO_3^- change (mmol·L^{-1}) scores across the resting and two experimental trials (4 km cycling TT). Although mean differences in peak values were between $\sim$ 0.8 and 0.9 mmol·L^{-1}, the range of difference scores across the three 0.3 g·kgBM^{-1} $NaHCO_3$ trials was over 3 mmol·L^{-1} within individuals at the high end (14), suggesting a large degree of intra-individual variability. The present study, using a combination of time-to-peak and TEM from the change scores (baseline to peak) over the 2 week pilot study (to objectively determine peak $sHCO_3^-$) still demonstrated a relatively large amount of variability across peak $sHCO_3^-$ values (0.6 mmol·L^{-1}; 95% CI 0.4 to 1.6 mmol·L^{-1}). Given the inter- (7, 8, 17) and intra-individual variability associated with identifying time-to-peak HCO_3^- values, our small but significant difference in performance times between trials, and assuming the athlete will replicate all dietary practices prior to competition, we recommend identifying the time course where an absolute increase of > 5 mmol·L^{-1} (HCO_3^-) occurs after ingesting 0.3 g·kgBM^{-1} $NaHCO_3$ as a criterion standard (2).

Despite implementing rigid 24 h dietary controls, a pre-race standardized ingestion strategy (10 ml·kgBM^{-1} fluid and 1.5 g·kgBM^{-1} CHO) based on previous best practice recommendations (20) and athletes' preferred pre-competition

diet, all athletes in the present study experienced at least some degree of GI distress, although the majority of it was minor (**Figure 5**). Regardless of severity, athletes will be reticent to use this supplement should it cause distraction during competition. Indeed, four of the top performing rowers in this study have not used $NaHCO_3$ during competition for this reason. Empirically, Saunders et al. have demonstrated improved exercise capacity when individuals do not experience GI discomfort after $NaHCO_3$ supplementation, albeit in recreationally trained participants (8). Given our participant cohort, we cannot speculate further as to whether the GI distress was directly related to the $NaHCO_3$, anxiety experienced by the rowers prior to the TT, the extreme intensity of the TT itself or a combination of factors. As suggested by numerous authors (2, 5, 6), GI distress associated with $NaHCO_3$ ingestion may be one of the primary factors in this supplement not reaching its ergogenic potential. Indeed, many contemporary studies have documented the commonly experienced side-effects (e.g., bloating, cramping, diarrhea, etc.) (8, 14, 18), and have even attempted to categorize these symptoms according to severity (16). However, as of yet there have been no direct causal links established between the severity of GI distress from $NaHCO_3$ ingestion and a decline in exercise performance. Moreover, the large disparity in the literature between ingestion protocols (e.g., capsules, liquid, fasted vs. fed-state), timing and nutritional control render between-study symptom comparison difficult. In the present study, although GI symptoms were similar regardless of condition, any opportunity to minimize the distractions associated with GI distress is logical and thus supports, where possible, prolonging the time period between ingestion and the start of competition.

One additional note in considering the ingestion strategy of the present study was the weight gain experienced by the athletes during both loading protocols. Although the BM increase was nominal for a weight supported sport such as rowing (<0.5%) and most likely a result of the 10 ml·kgBM^{-1} fluid administered during pill ingestion, it is worth mentioning given the potential for this supplement to be used in weight dependent (e.g., triathlon team relay, middle distance running etc.) and weight category sports (mixed-martial arts, boxing, etc.). Moreover, when separated from the group, the weight gain appeared more pronounced in the lightweight (72.5 kg) rowers ($\sim$ 0.6 kg increase; $n = 4$). To our knowledge, only one study has investigated the potential fluid increases after incorporating $NaHCO_3$ (ingested within the typical range) into a rehydration strategy to offset the effects of acute dehydration (12). Kupcis et al. observed a greater increase in BM during a 2 h loading sequence compared to the present study ($\sim$ 1.5 vs. 0.4 kg), albeit total fluid intake was over double the total volume (10 ml·kgBM^{-1} in the present study compared to 22 ml·kgBM^{-1}). However, these authors also did not observe any impact on performance (2,000-m rowing TT) compared to a nutrition/fluid-matched placebo control (12). Ultimately, when considering whether to implement a $NaHCO_3$ loading strategy it is worth noting that the expected body weight gain from $NaHCO_3$ ingestion is likely to be much less deleterious in weight supported (or independent) sports (rowing, canoe-kayak) than weight dependent sports (e.g., running, road cycling).

We acknowledge the perceived limitation in this study of not having a true placebo trial. However, the primary aim of this study was not to determine the efficacy of $NaHCO_3$ ingestion on 2,000-m rowing TT performance, as the potential ergogenic effect of this supplement has been shown previously across many published reviews and meta-analyses (2–5, 25, 29, 30). Rather, we sought to specifically address the question of whether or not ingestion timing impacts TT performance in an ecologically valid context (e.g., with dietary and warm-up conditions typically used by elite rowers). In an attempt to counterbalance our lack of a traditional placebo trial, deception was used to minimize the potential belief effect of timing (31). Thus, some level of expectancy was possible. When considering that coaching staff limited our time trial opportunities (i.e. two), and the fact that our time frame for carrying out the study was constrained, we agreed that the only way to mitigate the belief effect was to deceive the athletes about whether or not they were actually receiving $NaHCO_3$ in each trial. As nearly 65% of the athletes believed that the CON trial was indeed a placebo trial, we are confident this strategy was effective in "blinding" the athletes to the exact supplement they received. Given the limited peer-reviewed data available on this supplement in world-class athletes, and considering the inherent constraints of conducting research in a high performance environment (e.g., coach "buy-in," training and competition schedules only allowing for 2 × 2,000-m TT-tests), we felt conducting the study using the 60 min timing as a control for comparison against individual peak was warranted despite the somewhat non-traditional research design.

Ultimately, the findings of the present study may support targeting the onset of a competitive effort to coincide with an individual's peak blood buffering capacity window after $NaHCO_3$ supplementation if working with competitive athlete cohorts. Similarities in blood buffering changes after warm-up, however, would suggest this effect was not a result of any meaningful differences in blood alkalinity. Although a number of meta-analyses have suggested that ensuring the athlete has reached a minimum of a 5 mmol·L^{-1} absolute increase in blood [HCO_3^-] may also be important to maximize the effectiveness of this supplement (2, 26, 27), further study is required to directly test this threshold hypothesis. Regardless, understanding the total timeframe of this increase should allow for greater flexibility in the timing of $NaHCO_3$ supplementation (32). Moreover, given the extended time-frame above this 5 mmol·L^{-1} mark observed in all athletes (**Figure 2B**), we would recommend future research investigate the potential of this "window of opportunity" rather than focusing solely on peak blood buffering values. The flexibility in supplement timing across a potential performance window may help with individual GI issues, pre-competition food intake timing preferences and/or sport rules dictating warm-up time, check-in time or similar logistical constraints. In terms of supplement tolerance, even when incorporating a "tried and true" pre-race nutritional strategy that includes adequate fluid, carbohydrate and delayed ingestion timing, practitioners can expect some athletes to experience at least minor GI disturbances. Indeed, future work in this area may eventuate in eliminating this issue altogether (15, 32). Finally, those athletes participating in weight dependent sports and considering

$NaHCO_3$ supplementation should be conscious of acute increases in body weight associated with ingestion protocols designed to minimize GI distress.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Australian Institute of Sport Ethics Committee; approval code 20171205 and the University of Victoria Human Research Ethics Board; approval code 18-045. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SB, TS, GS and JS conceptualized and designed all aspects of the study. All authors contributed to data collection, analyses, writing of the manuscript, and agree to be accountable for the content of the work.

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Sodium Ingestion Improves Groundstroke Performance in Nationally-Ranked Tennis Players: A Randomized, Placebo-Controlled Crossover Trial

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This study examined the dose-response effects of ingesting different sodium concentrations on markers of hydration and tennis skill. Twelve British nationally-ranked tennis players (age: 21.5 ± 3.1 years; VO_{2peak}: 45.5 ± 4.4 ml·kg·min^{-1}) completed four identical in-door tennis training sessions in a cluster randomized, single-blind, placebo-controlled, crossover design. Twenty-minutes prior to each training session, participants consumed a 250 ml sodium-containing beverage (10, 20, 50 mmol/L) or a placebo (0 mmol/L), and continued to consume 1,000 ml of the same beverage at set periods during the 1-h training session. Tennis groundstroke and serve performance, agility, urine osmolality, fluid loss, sodium sweat loss and perceptual responses (rating of perceived exertion (RPE), thirst, and gastrointestinal (GI) discomfort) were assessed. Results showed that ingesting 50 mmol/L sodium reduced urine osmolality (−119 mOsmol/kg; $p = 0.037$) and improved groundstroke performance (5.4; $p < 0.001$) compared with placebo. This was associated with a reduction in RPE (−0.42; $p = 0.029$), perception of thirst (−0.58; $p = 0.012$), and GI discomfort (−0.55; $p = 0.019$) during the 50 mmol/L trial compared with placebo. Linear trend analysis showed that ingesting greater concentrations of sodium proportionately reduced urine osmolality ($\beta = -147$ mOsmol/kg; $p = 0.007$) and improved groundstroke performance ($\beta = 5.6$; $p < 0.001$) in a dose response manner. Perceived thirst also decreased linearly as sodium concentration increased ($\beta = -0.51$; $p = 0.044$). There was no evidence for an effect of sodium consumption on fluid loss, sweat sodium loss, serve or agility performance ($p > 0.05$). In conclusion, consuming 50 mmol/L of sodium before and during a 1-h tennis training session reduced urine osmolality and improved groundstroke performance in nationally-ranked tennis players. There was also evidence of dose response effects, showing that ingesting greater sodium concentrations resulted in greater improvements in groundstroke performance.

The enhancement in tennis skill may have resulted from an attenuation of symptomologic distracters associated with hypohydration, such as RPE, thirst and GI discomfort.

Keywords: exercise physiology, tennis, performance nutrition, sodium, fluid balance, hydration

INTRODUCTION

Tennis is a repetitive sprint sport, characterized by intermittent bouts of high-intensity exercise interspersed with periods of rest or low-intensity activity (1). The duration of a tennis match often exceeds 1 h and in some cases can last more than 5 h (2). Players also require a combination of fine and gross motor skills, agility and power to execute sport-specific tasks such as serves, groundstrokes, and volleys. Due to the high physiological demands, often under challenging environmental conditions, sweat losses during tennis match-play can be significant, with mean sweat rates of 0.6–2.6 L/h reported in the literature (3). This can lead to hypohydration (loss of body water) unless appropriate hydration strategies are implemented.

In addition to water, sweat contains substantial but variable amounts of sodium. Sweat sodium losses during tennis match-play are generally between 20 and 80 mmol/L (4–7). Excessive sodium lost through sweat, particularly when combined with over-drinking hypotonic beverages, reduces plasma sodium concentration leading to hyponatremia when plasma concentration is <135 mmol/L (8). This is problematic for athletes because sodium is an essential electrolyte that helps with fluid retention, cognition, muscle contraction, and nerve conduction (9–12), all of which are critical for the execution of technical skills in tennis.

The American College of Sports Medicine (ACSM) recommend that sodium should be ingested during exercise when large sweat sodium losses occur (13). The ACSM also suggest that sodium consumed in pre-exercise beverages may help athletes achieve euhydration prior to exercise (13). However, the evidence supporting this recommendation is limited. A recent systematic review (14) found that only one of the five included studies reported a significant benefit of sodium ingestion on endurance performance. In addition, four of the five included studies were conducted outdoors (14), meaning that the findings could have been confounded by factors such as ambient temperature, relative humidity, wind speed and direction. Furthermore, it is unknown whether sodium ingestion influences tennis-specific skill, which involves a combination of physical, technical, and cognitive factors.

There are currently no guidelines on the specific quantity of sodium ingestion required to optimize technical skills in sport, nor whether there is a dose-response relationship. Previous research has shown that consuming fluids with a higher sodium concentration reduces the occurrence of hyponatremia during prolonged exercise compared with fluids with a lower sodium concentration (15). However, this may not translate into improved sports performance (15). There is a need for further well-controlled studies to inform sports nutritional guidelines and sodium replacement strategies. Therefore, the primary purpose of this study was to examine the dose-response effects of ingesting different sodium concentrations on hydration status, fluid balance, agility, perceptual responses and tennis skill performance in British nationally-ranked tennis players.

MATERIALS AND METHODS

Study Design

This study used a cluster randomized, single-blind, placebo-controlled, crossover design. Participants completed two familiarization trials and an incremental cardiopulmonary exercise test (CPET), followed by four identical tennis training sessions that were separated by 7 days. Twenty-minutes prior to each tennis training session, participants consumed a 250 ml sodium-containing beverage (10, 20, 50 mmol/L) or a placebo, and continued to consume 1,000 ml of the same beverage at set periods during the training session. The order of the beverages was randomized but was not counter-balanced i.e. all participants received the beverages in the same order. Body mass (kg) and urine osmolality (UOsm) were measured immediately before and after training sessions to assess fluid loss and hydration status, respectively. Groundstroke and serve performance, agility, rating of perceived exertion (RPE), thirst, and gastrointestinal (GI) discomfort were recorded during the training sessions, whilst sweat sodium concentration and total sweat sodium loss were assessed immediately afterwards.

Participants

Twelve nationally-ranked tennis players [mean (SD) age: 21.5 ± 3.1 years; body mass: 71.5 ± 7.1 kg; height: 178 ± 5 cm; peak oxygen consumption (VO_{2peak}): 45.5 ± 4.4 $ml \cdot kg \cdot min^{-1}$] were recruited from the University of Hull Men's 1st Tennis Team and volunteered to take part in this study. All participants were currently ranked between 50 and 1,000 in Great Britain based on Lawn Tennis Association (LTA) national rankings, and regularly competed in LTA Grade 3 tournaments (high-level regional standard). Participants were part of the same training group and informed of the experimental procedures prior to signing an institutionally approved informed consent document to participate in the study. Ethical approval for the study was granted by the Sports, Health and Exercise Science Ethics Committee at the University of Hull.

Procedures

Familiarization Sessions and Cardiopulmonary Exercise Test (CPET)

The week prior to the first experimental training session, participants completed two familiarization trials and an incremental CPET on separate days. Participants initially completed a medical questionnaire and had their body mass, height, resting blood pressure and heart rate recorded. The incremental CPET was then performed on a motorized

treadmill (Cosmos Pulsar, H/P Cosmos, Nussdorf-Traunstein, Germany) to characterize participants' cardiopulmonary fitness at baseline. The CPET protocol began with a treadmill speed of 8 $km \cdot h^{-1}$ at a constant incline of 1.5%, which increased in speed every 3-min by 2 $km \cdot h^{-1}$ respectively, until volitional exhaustion. Exhaustion was defined as an inability to maintain the required running speed despite strong verbal encouragement. Breath-by-breath data (Oxycon Pro, CareFusion, Hoechberg, Germany) were recorded throughout and averaged per minute before interpretation. VO_{2peak} was determined as the highest VO_2 attained during the final 30-s of the CPET. The familiarization sessions were identical to the tennis training sessions apart from that no data were collected and no restrictions were implemented.

Tennis Training Session

Participants consumed 500 ml of water 4-h before each tennis training session to ensure they started in a euhydrated state. All training sessions lasted 1-h and took place across two indoor tennis courts inside a three-court facility. Participants were instructed not to perform moderate- to vigorous-intensity exercise for at least 48 h prior to each experimental session. Sessions were performed at the same time of day (7:00 p.m.), each separated by exactly 7 days. Ambient temperature ($16.5 \pm 1.1°C$) and humidity ($30 \pm 7.6\%$) were maintained using the indoor air conditioning unit and were monitored remotely throughout using a wireless weather station (BAR816HG, Oregon Scientific, Tualatin, Oregon). This reflects the climate set in UK indoor tennis facilities. Participants wore only a t-shirt, shorts, socks, and shoes during the training sessions. Participants were provided with a 3-min break every 15-min throughout each training session, to allow them to consume the sodium-containing beverage (or placebo).

Training sessions began with a standardized 10-min dynamic warm-up, followed by a 15-min tennis-specific warm-up, prior to the main training session. The tennis-specific warm-up consisted of paired stroke shot rallying (2-min), volleys (1-min), and serve practice (2-min) across half a court, followed by 2 × 5-min half-court matches. Standard tennis rules applied during the half-court games, apart from that players could use the tram-lines during the point. The same opponents were used in each training session, which were matched as closely as possible based on their current LTA ranking. The stroke performance test involved two technical tennis drills; groundstrokes and serves. First, participants hit 20 forehands and 20 backhands cross-court, aiming for 3×3 m^2 target areas in both opposite corners of the court, which were marked with lines. They performed four shots (2 forehands and 2 backhands), jogged round to collect the four balls and returned for another four shots until 40 had been completed. Participants were encouraged to perform "attacking" shots to the best of their ability and performed the drill in the same order each time, waiting at the back of the court until it was their turn to perform the drill. An LTA qualified coach was stood on the opposite side of the court at the net and threw the ball underarm to the participants in the same place for the forehand and backhand (in the corner between the base-line and tram-line) marked out by a 1×1 m^2 lined target. After a player sprinted to perform a shot, they were allowed 2 s to return to the center of the baseline ready to perform the next shot emphasizing the need for a quick acceleration and deceleration before and after each shot. The ball bounced once before being struck by the participant. This drill was performed on two tennis courts with six participants on each court. The same coaches threw the balls for each participant during all experimental sessions. Participants then performed 10 serves from the right and 10 serves from the left, aiming for a 2×4.6 m^2 area at the top of both service boxes marked with lines. They were allowed 30 s between serve attempts. To minimize learning effects, the tennis training session involved drills that the participants were highly accustomed to performing during prior routine training sessions. After finishing the tennis drills, participants completed a Pro-Agility test (see outcome measures below).

Sodium-Containing Beverages

Twenty-minutes before the start of each experimental session, participants consumed a 250 ml beverage containing either 0 (placebo), 10, 20, or 50 mmol/L of sodium (Light Gray Celtic Sea Salt, Selina Naturally, Arden, NC) in a cluster randomized, non-counterbalanced order. Participants then continued to consume 1,000 ml of the same beverage at set 3-min intervals every 15-min during the training session. These intervals were chosen to replicate the frequency of rest intervals in competition. The volume of fluid consumed during the 3-min intervals was *ad libitum*, but participants were instructed to consume the entire 1,000 ml beverage by the end of the session, which was confirmed by a research team member. The order of the experimental sessions for all participants was: 10, 50, 0, and 20 mmol/L. Commercially available sports drinks generally provide ∼20 mmol/L of sodium per serving (16). Hence, the concentrations of sodium used in this study reflect lower, similar and higher concentrations for comparison. The flavor of each solution was masked with zero-calorie flavoring (Flavdrops™, MyProtein, Warrington, UK), and participants were blind to the beverage they received. As per the participant information sheet, participants were told that the researchers were testing the effect of four different sports drinks on performance. They were informed that the highest sodium concentration of any drink was 50 mmol/L, but they were unaware that the sodium content of the drink differed nor that the researchers were specifically testing for the effects of sodium. All participants confirmed that they did not know which beverage they were receiving after completing the study. They were not permitted to spit out any of the liquid or pour it onto their hair or face. Beverages in all four experimental conditions were made with the same brand and batch of still water (ASDA Still Natural Mineral Water, Asda, Leeds, UK) to ensure consistency of the water mineral content. The mineral concentration of the water was as follows: Calcium, 0.23 mmol/L; Magnesium, 0.08 mmol/L; Potassium, 0.051 mmol/L; and Sodium, 0.4 mmol/L. The sodium concentration of each beverage was confirmed prior to training sessions using the B-722 LAQUAtwin Sodium Ion Meter (Horiba, Kyoto, Japan), which was calibrated before every

session using a standard solution containing 150 parts per million (ppm) of sodium.

Dietary Analysis

Participants completed a 48-h record of their food and fluid intake before each tennis training session. We instructed participants to maintain their habitual, eucaloric diets throughout the study period, and to consume the same foods/fluids for 48-h prior to each session. Participants also refrained from consuming alcohol or caffeine for 48-h before training sessions. Diet records were analyzed for sodium content using the smartphone application MyFitnessPal (Under Armor, Baltimore, MD).

Outcome Measures

Hydration Status

Change in hydration status from pre- to post-session was determined with UOsm. After consuming the 250 ml beverage 20-min prior to the tennis training session, participants emptied their bladders and provided a midstream urine sample directly into 30 ml clear, plastic, sterile container. A second sample was collected post-session. Urine samples were assessed for UOsm within 10-min of collection using a portable osmometer (Osmocheck, Vitech Scientific, West Sussex, UK) that has previously been validated (17). The osmometer provides a measurement range of 0–1,500 mOsmol/kg and was calibrated using distilled deionised water before each session.

Sweat Sodium Concentration

Immediately before participants began the tennis training sessions, four absorbent sweat pads (8 × 6 cm; Adhesive Dressing, Boots, Nottingham, UK) were applied to four sites of the body: (1) on the posterior midline of the right forearm, equidistant between the antecubital fossa and wrist joint, (2) the midline of the widest circumference of the right calf, (3) on the anterior midline of the right thigh, equidistant between the patella and greater trochanter, and (4) 5-centimeters lateral from the third lumbar spine vertebra (L3). These sites were chosen to align with previous research (5) and because they are highly correlated to whole-body sweat sodium loss (18) and provide minimum disruption of tennis performance (5). Prior to application, each patch site was shaved with a handheld razor, cleaned using an alcohol wipe, washed with deionised water and dried with a clean, electrolyte free swab gauze (Boots). Immediately post-session, the sweat pads were removed using a pair of sterile forceps (a new pair for each patch) and individually placed inside the barrel of a 10-ml plastic syringe (King Scientific, Liversedge, UK). Deionised water (5-ml) was added to the patch to ensure a sufficient volume of solution was obtained for analysis. The syringe plunger was then depressed to compress the pad and obtain a minimum 5-ml sample of sweat. This sample was immediately analyzed for sodium concentration (mmol/L) by the B-722 LAQUAtwin Sodium Ion Meter, which corrected for the 5 ml of deionised water previously added. This method is a valid field technique of estimating sodium concentration (19). Patches were not assessed for background sodium. Whole-body sweat sodium concentration was determined through a validated area weighted mean of four skin regions (18) as adapted by previous tennis sweat sodium research (5, 18). Mean whole-body sweat sodium concentration = 28.2% calf + 28.2% scapula + 11.3% forearm + 32.2% thigh.

Fluid and Sweat Sodium Loss

Fluid loss was estimated from the pre-post change in body mass after correcting for the 1,000 ml of fluid consumed throughout the 1-h training session. Body mass was measured to the nearest 0.1 kg using a calibrated digital scale (seca 813, SECA, Birmingham, UK). Participants wore only underwear and removed all jewelry. Body mass was then re-assessed within 5-min of the conclusion of the training session, before participants had urinated/defecated/consumed food or liquid, and after sweat pads had been collected from the skin sites and participants thoroughly towel dried their bodies. In line with previous research (5, 18, 20, 21), loss of mass was not corrected for respiratory water loss or loss due to substrate exchange because this would be impractical for practitioners to include when assessing fluid requirements (22). Total sweat sodium loss (g) was calculated by multiplying the volume of fluid loss (L) by the molecular mass of sodium (22.99 g/mol) and the concentration of whole-body sweat sodium loss (mmol/L) (5).

Perceptual Responses

Rating of perceived exertion (RPE), gastrointestinal (GI) discomfort, and perceived thirst data were collected during each 15-min break during the 1-h training session and after the session had finished (total of 4 times). A 10-point Borg scale (arbitrary units [AU]) was used to collect RPE data (23). Thirst and GI discomfort were also rated on a 10-point Likert scale (AU) ranging from "No discomfort" and "Not thirsty" to "Unbearable" and "Excessive thirst," respectively. Participants were formally familiarized with the scales during the two prior familiarization sessions. The four scores in each outcome were averaged prior to analysis to reduce the number of statistical comparisons made.

Stroke Performance

The number of forehand strokes, backhand strokes, and serves to land in the pre-specified target areas during the tennis training session were recorded. Forehand and backhand stroke scores were combined to provide an overall tennis groundstroke score (maximum of 40). A point was given if any part of the ball touched the line encompassing the target area. The drills were recorded by a 50 Hz video camera (Panasonic Lumix DC-TZ200), with the videos being viewed post-session to determine the number of successful attempts.

Pro-Agility Test

Agility performance was assessed with the Pro-Agility test. A distance of 9.14 m was measured and marked with a line on the tennis court. Participants began in a neutral 3-point position with feet placed equally either side of the midline. After a countdown of "one, two, three, go" by the researcher, participants turned and ran 4.57 m to their left and touched the line with their left hand, then ran 9.14 m to the right whilst touching the line with their right hand, then ran back 4.57 m through the midline. Time began with initial movement and ended when the participant

TABLE 1 | Descriptive statistics of outcomes in each experimental condition (mean ± SD or median [IQR]).

	50 mmol/L	20 mmol/L	10 mmol/L	Placebo	Main effect of condition (*p*)
Body mass (kg)					
Pre-trial	71.9 ± 7.6	71.9 ± 7.2	72.5 ± 7.3	72.1 ± 7.1	–
Post-trial	72.5 ± 7.5	72.3 ± 7.1	72.9 ± 7.3	72.5 ± 7.1	–
Change	0.55 ± 0.14	0.48 ± 0.22	0.45 ± 0.18	0.37 ± 0.15	0.08
Fluid loss (L/hour)[a]	−0.45 ± 0.14	−0.52 ± 0.22	−0.55 ± 0.18	−0.63 ± 0.15	0.08
Urine osmolality (mOsmol/kg)					
Pre-trial	635 [500, 745]	490 [380, 743]	485 [338, 663]	405 [210, 743]	–
Post-trial	395 [345, 610]	430 [388, 723]	480 [368, 708]	645 [388, 758]	–
Change	−210 (−308, −48)*	−25 (−105, 63)	55 (10, 185)	90 (−40, 178)	0.015
Sweat Na^+ (mmol/L)					
Whole-body	69.7 ± 9.4*	67.3 ± 8.6*	54.9 ± 8.0*	61.3 ± 11.1	<0.001
Total sweat Na^+ loss (g)[b]	−0.73 ± 0.30	−0.79 ± 0.36	−0.70 ± 0.27	−0.89 ± 0.26	0.37
Perceptual responses					
RPE	3.2 ± 1.1*	3.9 ± 0.7	3.8 ± 1.1	3.5 ± 1.1	0.019
GI discomfort	2.4 ± 0.9*	3.6 ± 1.2	3.1 ± 0.8	2.6 ± 0.9	0.002
Thirst	2.8 ± 0.9*	3.8 ± 0.7	3.0 ± 0.8	3.8 ± 1.2	0.001
Tennis skill and agility					
Successful stroke attempts	22.2 ± 5.7*	15.3 ± 4.8	16.3 ± 4.6	13.5 ± 5.0	<0.001
Successful serve attempts	10.8 ± 2.4	10.6 ± 1.7	10.6 ± 2.8	10.5 ± 2.2	0.99
Pro-Agility test (s)	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.2	4.5 ± 0.1	0.11

GI = gastrointestinal; p = p-value; RPE = rating of perceived exertion.

[a] *Fluid loss was calculated as: pre-post change in body mass (kg)−1,000 (ml) to correct for the 1,000 ml of fluid consumed at set periods throughout the 1-h tennis training session.*

[b] *Total sweat sodium loss was calculated as the product of fluid loss, the molecular mass of sodium, and whole-body sweat sodium (Na^+) concentration.*

**Statistically different from placebo ($p < 0.05$).*

crossed the midline a second time, covering a total distance of 18.28 m. Two researchers recorded time with a stopwatch, with the average time recorded to the nearest to the nearest 0.01-s. Three trials were performed, separated by 3-min of rest, with the fastest time used for analysis. Participants completed the test in the same order during each session.

Statistical Analyses

Data were analyzed in R (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics are reported as mean ± SD or median (interquartile range). Paired *t*-tests with a Bonferroni correction were used to compare pre-trial UOsm and body mass between conditions. Differences in study outcomes between conditions were assessed with a multilevel linear model. Condition was entered into the model as a fixed factor with four levels (0, 10, 20, or 50 mmol/L sodium) and participants were entered as a random factor with individual intercepts. For pre-post changes in UOsm and body mass, the pre-session value was also entered into the model as a covariate. If there was a significant main effect of condition (i.e. if the inclusion of "condition" in the model significantly improved the model fit), the data were further explored using three non-orthogonal contrasts; where the 10, 20, and 50 mmol/L conditions were separately compared to the placebo condition. The contrasts were adjusted for multiple comparisons using a Bonferroni correction, and mean differences with their 95% confidence intervals (CIs) from the comparisons are presented. Subsequently, we conducted a linear trend analysis using the stats::contr.poly() function in R to investigate whether there was a linear dose response effect. Statistical significance was set at a two-tailed $p < 0.05$.

RESULTS

Descriptive statistics are presented in **Table 1**. For outcomes with a significant main effect of condition, follow-up comparisons and linear trend analyses are presented in **Table 2**.

Hydration Status and Fluid Loss

There were no significant differences in pre-trial UOsm or body mass between conditions ($p > 0.05$; **Table 1**), which suggests that participants were in a similar hydrated state before each trial. There was no significant effect of condition on pre-post change in body mass ($p = 0.076$) or fluid loss ($p = 0.080$). However, there was a significant main effect of condition for the pre-post change in UOsm (**Table 1**). Contrasts showed that the reduction in UOsm was significantly greater in the 50 mmol/L trial compared with placebo (−119 [−227, −11.9] mOsmol/kg, $p = 0.037$). Furthermore, trend analysis showed a significant linear relationship between sodium ingestion and UOsm, demonstrating that as the dose of sodium increased, UOsm decreased proportionately ($\beta = -147$ [−248, −45.6] mOsmol/kg; $p = 0.007$).

TABLE 2 | Mean differences (95% CI) in outcomes between experimental conditions.

	50 mmol/L vs. placebo		20 mmol/L vs. placebo		10 mmol/L vs. placebo		Linear trend	
	Mean (95% CI)	***p***	**Mean (95% CI)**	***p***	**Mean (95% CI)**	***p***	**β (95% CI)**	***p***
Change in urine osmolality (mOsmol/kg)	−119 (−227, −11.9)	0.037	−11.0 (−116, 94.2)	1.0	51.0 (−54.2, 156)	0.76	−147 (−248, −45.6)	0.007
Whole-body Sweat Na^+ (mmol/L)	6.4 (3.0, 9.9)	<0.001	4.0 (0.51, 7.5)	0.028	−8.4 (−11.9, −4.9)	<0.001	8.5 (4.3, 12.6)	<0.001
RPE	−0.42 (−0.78, −0.05)	0.029	0.33 (−0.03, 0.70)	0.11	0.18 (−0.19, 0.54)	0.77	−0.18 (−0.57, 0.20)	0.35
GI discomfort	−0.55 (−1.0, −0.09)	0.019	0.66 (0.20, 1.1)	0.005	0.18 (−0.28, 0.64)	1.0	−0.07 (−0.60, 0.45)	0.79
Thirst	−0.58 (−1.0, −0.13)	0.012	0.45 (0.00, 0.90)	0.062	−0.33 (−0.77, 0.12)	0.26	−0.51 (−1.0, −0.03)	0.044
Groundstroke performance	5.4 (3.1, 7.6)	<0.001	−1.5 (−3.8, 0.72)	0.33	−0.54 (−2.8, 1.7)	1.0	5.6 (3.3, 7.9)	<0.001

95% CI = 95% confidence interval; GI = gastrointestinal; p = p-value; RPE = rating of perceived exertion.

Sweat Sodium Concentration

There was a significant main effect of condition on whole-body-sweat concentration (**Table 1**). Both the 50 mmol/L (6.4 [3.0, 9.9] mmol/L; $p < 0.001$) and 20 mmol/L (4.0 [0.51, 7.5] mmol/L; $p = 0.028$) trials reported significantly higher whole-body sodium sweat concentrations compared with placebo (**Table 2**), and there was a significant linear trend between condition and whole-body sweat sodium concentration (β = 8.5 [4.3, 12.6] mmol/L; $p < 0.001$). However, there was no significant effect of condition on total sodium sweat loss ($p = 0.37$). Dietary sodium intake in the 48-h before each trial was not different between conditions (50 mmol/L = 4.4 ± 1.5 g; 20 mmol/L = 3.7 ± 0.66 g; 10 mmol/L = 3.8 0 ± 0.89 g; placebo = 4.0 ± 1.2 g; $p = 0.20$).

Tennis Skill and Agility Performance

Groundstroke, serve, and agility performance are presented in **Table 1** and **Figure 1**. There was a significant main effect of condition for groundstroke performance, with follow-up contrasts showing that groundstroke performance was significantly greater during the 50 mmol/L trial compared with placebo (5.4 [3.1, 7.6]; $p < 0.001$). Further, there was a significant linear relationship between sodium concentration and stroke performance, demonstrating a dose response effect (β = 5.6 [3.3, 7.9]; $p < 0.001$). There was no significant effect of condition on serve ($p = 0.99$) or agility ($p = 0.11$) performances.

Perceptual Responses

There was a significant main effect of condition on all perceptual responses. Perceived thirst (−0.58 [−1.02, −0.13]; $p = 0.012$), GI discomfort (−0.55 [−1.01, −0.09]; $p = 0.019$), and RPE (−0.42 [−0.78, −0.05]; $p = 0.029$) were all significantly lower in the 50 mmol/L trial compared with placebo (**Table 2**). Trend analysis also showed that as the beverage sodium increased, perceived thirst decreased in a linear manner (β = −0.51 [−1.00, −0.03]; $p = 0.044$). GI discomfort was greater in the 20 mmol/L trial compared with the placebo condition (0.66 [0.20, 1.11]; $p = 0.005$).

DISCUSSION

This is the first study to evaluate the effectiveness of consuming different sodium concentrations on markers of hydration and tennis skill performance in nationally-ranked tennis players. The main findings were that 50 mmol/L of sodium ingestion before and during a 1-h tennis training session reduced UOsm and improved groundstroke performance compared with placebo. There was also evidence of dose-response effects, demonstrating that ingesting greater concentrations of sodium promoted proportionately greater improvements in hydration status and groundstroke performance. These results provide novel evidence for sodium consumption as an effective nutritional strategy for enhancing tennis skill.

It is well-established that hypohydration reduces aerobic capacity and cognitive function (24–26). Given that tennis skills are dependent on a combination of physical and cognitive factors, hypohydration may also account for impaired technical skill (3). Indeed, whilst research in tennis is scarce, studies with basketball (27, 28) and soccer players (29) have reported impaired execution of sport-specific skills, although this is not always the case (30, 31). A novel finding of our study was that there was a dose-dependant increase in the effects of sodium on tennis performance in a randomized placebo-controlled, crossover trial. Ingesting 50 mmol/L of sodium before and during a tennis training session improved groundstroke performance compared with placebo. Consuming 50 mmol/L sodium also resulted in a greater UOsm reduction from pre- to post-trial compared with placebo (-119 mOsmol/kg), which is indiciative of a change in hydration status. Accordingly, using UOsm ≥ 700 mOsmol/kg to define hypohydration (25), four of 12 participants in the placebo trial were considered hypohydrated post-session, compared with only one participant in the 50 mmol/L sodium trial. Thus, our findings suggest that the improved groundstroke performance following 50 mmol/L of sodium ingestion was underpinned by changes in UOsm.

Although urine volume was not measured in this study, which could be considered a limitation, it was expected that the 50 mmol/L trial would have produced lower urine volume thus increased UOsm (32). Pre-trial UOsm was insignificant between trials, but values increased numerically higher. As the pre-trial urine sample was collected 20-min after the initial 250 ml bolus had been consumed, the placebo drink may have increased urine output and produced a more dilute urine concentration, potentially affecting the difference between pre-post exercise UOsm. Therefore, measurement of urine volume should be

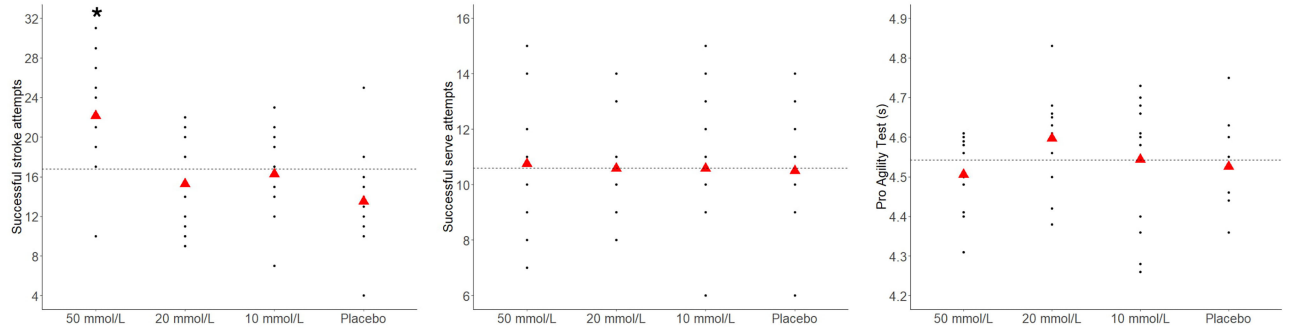

FIGURE 1 | Groundstroke **(A)**, serve **(B)**, and agility **(C)** performance during each experimental condition. The dashed horizontal line represents the mean score across all conditions. *Significantly different to placebo ($p < 0.001$).

considered in future studies. Furthermore, the 50 mmol/L trial showed the highest pre-trial UOsm reading compared to all other trials (635 mOsmol/kg), suggesting the 50 mmol/L drink could have had a greater hydrating effect due to differences in baseline values. Nevertheless, this difference in UOsm at baseline did not reach statistical significance, and we controlled for differences in baseline values and regression to the mean effects by including the baseline scores as a covariate in the statistical model.

Trend analyses provided evidence for dose-response effects of sodium ingestion on hydration and groundstroke performance. That is, as the concentration of sodium increased from placebo to 10, 20, and 50 mmol/L, UOsm changes and groundstroke performance improved linearly. This result provides further support for our findings and the use of sodium as an ergogenic aid in tennis, and warrants further research evaluating the optimal dose of sodium to enhance sport performance. Sodium consumption may improve hydration status by increasing plasma sodium concentration, which helps retain ingested fluid via osmotic processes. Improved hydration status has a direct effect on physical performance by maintaining plasma volume, body core temperature, muscle perfusion and muscle metabolism, amongst other mechanisms (26, 32). In contrast, there appears to be no clear physiological mechanism by which modest improvements in hydration status might improve sport skill or cognitive function (32). Instead, improved hydration may attenuate the distracting symptoms of hypohydration, such as negative mood state (33), thirst (33), GI complaints (34), and increased RPE (35). In line with this supposition, our findings showed that consuming 50 mmol/L sodium significantly reduced RPE, perceptions of thirst and GI discomfort compared to placebo. Trend analysis also showed that as the concentration of sodium increased, perceived thirst significantly decreased in a linear manner. Sodium consumption may increase osmotic material retained in the extracellaur space, increasing blood osmolality and thirst perception (36) The trials in this study provided sufficient fluid to increase pre- to post-session body mass with research suggesting thirst is stimulated following a 1–2% body mass loss (37, 38). Thus, taken together with previous research, the improved groundstroke performance induced by sodium consumption may have resulted from a combined attenuation of symptomologic distracters such as thirst, GI distress, and RPE instead of a direct physiological effect of hydration and warrants further investigation.

Despite the observed improvement in groundstroke performance, there was no evidence for an effect of sodium ingestion on serve performance. The reason for this is unclear but may be due to the fact that tennis serving is pre-planned and does not require the player to react to a stimulus, whereas the groundstroke task involved reacting to tennis balls being thrown in quick succession (2-s) by a LTA coach. The groundstroke task therefore encompassed greater cognitive components (including visual scanning, reaction time, and decision making), which may have improved through sodium ingestion.

Fluid lost during the control trial in this study (0.63 L/h, 0.9% of initial body mass) was mild compared to the range of mean fluid losses reported in the literature (0.6–2.6 L/h) (3). This is likely due to the indoor environment, the ambient temperature (16.5 ± 1.1°C), the duration of training sessions (60-min), and the protocol involving training and not match-play (39). It has been proposed that ≥2% fluid loss represents the threshold for reductions in endurance performance (32). However, impaired cognition has been observed at fluid losses of <1% (40, 41), which supports the assertion that the modest degree of hypohydration observed in the placebo condition (0.9% fluid loss) could have impaired cognitive function, probably as a byproduct of the aforementioned distracting symptoms, and subsequently reduced tennis skill.

Despite observing a statistically significant difference in UOsm between the 50 mmol/L sodium and control trials, there was no evidence for an effect of sodium ingestion on fluid loss ($p = 0.08$) or total sodium sweat loss ($p = 0.37$). Discrepancies between UOsm and fluid losses have been reported previously (42). Although evaluating fluid losses via

changes in body mass aligns with previous research and is a suitable field-based technique (3, 5, 18, 20, 32, 43), there are sources of error associated with this method, including respiratory water loss and loss due to substrate exchange (22). Water produced during substrate oxidation, release of water previously bound with glycogen, and accumulation of water in the bladder also cause a dissociation between body mass changes and hydration status (22, 44). Thus, given the potential for error and the small fluid losses observed in this study, monitoring changes in body mass might not have been sensitive enough to detect subtle differences in hydration status between conditions.

There was no evidence of an effect of sodium ingestion on agility performance. Whilst limited data exist, previous research with soccer players suggests that hydration status does not influence 15-m sprint performance (30, 45). Thus, consuming sodium to mitigate the effects of hypohydration would not be expected to improve performance in the Pro-Agility test. It is important to note that the Pro-Agility test does not require players to react to a stimulus, which limits the cognitive requirements of the task. Hoffman et al. (46) reported improved lower-body reaction time when basketball players drank water during a 40-min game compared with no fluid (0 vs. 2.3% body mass loss). Hydration status might differently affect performance in pre-planned vs. reactive agility tasks. Alternatively, greater fluid loss than observed in this study (0.9% of body mass) and thus greater levels of hypohydration may be required to influence such tasks. Further research should aim to determine whether sodium ingestion improves reactive agility, which more closely replicates tennis match-play.

A limitation of this study was that research team members were not blind to the type of beverage that participants received, although the researchers strictly adhered to a pre-determined protocol. We used cluster randomization to randomize the order of the experimental sessions, with the unit of randomization being the whole sample of participants (order: 10, 50, 0, 20 mmol/L), which may have introduced systematic bias. However, there was no evidence of an order effect on the outcome measurements given that we found a linear dose response on UOsm and groundstroke performance as the dose of sodium increased from 0 to 10, 20, and 50 mmol/L. Although dietary sodium intake was measured, along with consumption of 250 ml of the sodium solution 20-min prior to testing, this may not have allowed enough time to affect pre-trial UOsm readings, affecting baseline hydration scores. Furthermore, whilst participants were blind to the sodium concentration of the beverages and the flavor was masked, we cannot guarantee that participants were not aware of the relative sodium content of the beverages, although participants anecdotally reported that they could not identify any differences between the drinks. Finally, this study also used a relatively small sample size ($n = 12$), which limits the precision of the effect estimates.

In conclusion, consuming 50 mmol/L of sodium before and during a 1-h tennis training session reduced UOsm and improved groundstroke performance in nationally-ranked tennis players. There was also evidence of dose-response effects, showing that ingesting greater sodium concentrations resulted in greater improvements in hydration and groundstroke performance. The observed enhancement in tennis skill may have resulted from an attenuation of symptomologic distracters associated with hypohydration, such as thirst, GI discomfort, and RPE. Practitioners and sports nutritionists should include sodium ingestion in their nutritional arsenal as a simple and effective strategy to improve tennis-specific skill.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Sports, Health and Exercise Science Ethics Committee at the University of Hull. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EM, JB, ST, PM, and RV were responsible for the conceptualization and formulation of the overarching research question, as well as the development and design of study methodology. EM and JB were principally responsible for data collection. SO was responsible for data curation, statistical analyses, and writing the original draft of the manuscript. PM and RV were responsible for the overall oversight and leadership of the study. All authors reviewed, edited, and approved the final version of the manuscript.

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Capsule Size Alters the Timing of Metabolic Alkalosis Following Sodium Bicarbonate Supplementation

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Introduction: Sodium bicarbonate ($NaHCO_3$) is a well-established nutritional ergogenic aid that is typically ingested as a beverage or consumed in gelatine capsules. While capsules may delay the release of $NaHCO_3$ and reduce gastrointestinal (GI) side effects compared with a beverage, it is currently unclear whether the capsule size may influence acid–base responses and GI symptoms following supplementation.

Aim: This study aims to determine the effects of $NaHCO_3$ supplementation, administered in capsules of different sizes, on acid–base responses, GI symptoms, and palatability.

Methods: Ten healthy male subjects (mean ± SD: age 20 ± 2 years; height 1.80 ± 0.09 m; weight 78.0 ± 11.9 kg) underwent three testing sessions whereby 0.3 g $NaHCO_3$/kg of body mass was consumed in either small (size 3), medium (size 0), or large (size 000) capsules. Capillary blood samples were procured pre-ingestion and every 10 min post-ingestion for 180 min. Blood samples were analyzed using a radiometer (Radiometer ABL800, Denmark) to determine blood bicarbonate concentration ($[HCO_3^-]$) and potential hydrogen (pH). GI symptoms were measured using a questionnaire at the same timepoints, whereas palatability was recorded pre-consumption.

Results: Capsule size had a significant effect on lag time (the time $[HCO_3^-]$ changed, T_{lag}) and the timing of peak blood $[HCO_3^-]$ (T_{max}). Bicarbonate T_{lag} was significantly higher in the large-sized (28 ± 4 min) compared with the small-sized (13 ± 2 min) capsules ($P = 0.009$). Similarly, T_{max} was significantly lower in the small capsule (94 ± 24 min) compared with both the medium-sized (141 ± 27 min; $P < 0.001$) and the large-sized (121 ± 29 min; $P < 0.001$) capsules. The GI symptom scores were similar for small-sized (3 ± 3 AU), medium-sized (5 ± 3 AU), and large-sized (3 ± 3 AU) capsules, with no significant difference between symptom scores ($F = 1.3$, $P = 0.310$). Similarly, capsule size had no effect on palatability ($F = 0.8$, $P = 0.409$), with similar scores between different capsule sizes.

Conclusion: Small capsule sizes led to quicker T_{lag} and T_{max} of blood [HCO_3^-] concentration compared to medium and large capsules, suggesting that individuals could supplement $NaHCO_3$ in smaller capsules if they aim to increase extracellular buffering capacity more quickly.

Keywords: buffering, gastrointestinal disturbance, performance, acid base balance, palatability

INTRODUCTION

Sodium bicarbonate ($NaHCO_3$) is an extensively researched nutritional ergogenic aid shown to be particularly effective in improving short-duration (∼1–10 min), high-intensity exercise performance (1–3). Supplementation with $NaHCO_3$ serves to enhance endogenous bicarbonate buffering capacity by inducing temporary elevations in extracellular bicarbonate concentrations and, resultantly, enhancing the efflux of hydrogen cations (H^+) from the skeletal muscle. Consequently, an improved H^+ efflux attenuates muscular fatigue and has been shown to positively impact multiple performance measures such as total work done (4), power output (5), and time to exhaustion (6) and recovery between exercise bouts (7).

The ergogenic potential of $NaHCO_3$ is widely acknowledged (8), but some individuals suffer adverse gastrointestinal symptoms (9, 10) (GIS) that may be deleterious to performance (11, 12). Recently, some authors have attempted to find strategies to alleviate the severity of GIS by using delayed release (2019) and enterically coated capsules (13). This strategy builds on the concept of reducing GIS by delaying the release of HCO_3 into the stomach, thereby limiting carbon dioxide production that occurs when $NaHCO_3$ is ingested (14, 15). At present, these coatings make this ergogenic strategy expensive. Indeed the most frequently used ingestion strategy is gelatine capsule delivery of $NaHCO_3$. This is both a cheap alternative, improves the palatability compared to the traditional solution, and widely used by athletes and researchers.

Encapsulation may result in reductions in the HCO_3 lost in the stomach and bring about comparable acid–base changes using smaller doses than required from aqueous delivery (15). There is, however, a suggestion that encapsulation may impair or slow down bicarbonate availability through decreased gut transit time (16) changing the optimal pre-exercise ingestion time. Additionally, while the gastro-resistant properties of different capsule forms and their subsequent effects on bicarbonate bioavailability have begun to be elucidated (10, 13, 17), the effects of the physical properties of capsules, such as their overall size (and therefore surface area), on bicarbonate bioavailability remain unclear.

In the pharmaceutical industry, the bioavailability of a substance is carefully considered as part of delivery vehicle testing and is affected by size, surface area, and surface area/volume of the capsule. Furthermore, there is a direct relationship between the surface area of a substance and its dissolution rate; specifically, an increase in total surface area of a delivery vehicle in contact with the gastrointestinal fluids causes an increase in the dissolution rate (18). Indeed the dissolution of substances from capsules is a complex function of four key factors: (1) the rate of dissolution of the capsule shell, (2) the rate of penetration of gastrointestinal fluids into the gastrointestinal mass, (3) the rate at which the mass disaggregates in the gastrointestinal fluids, and (4) the rate of dissolution of the dispersed substance particles (18). Such factors are rarely considered in the delivery of ergogenic aids despite these processes being highly variable and subject to potentially large inter-individual variation (19). Given the considerable evidence of the ergogenic effects of $NaHCO_3$ and the widespread use of capsules as an ingestion strategy, understanding how capsule size (and therefore surface area) impacts bioavailability is of high importance for optimizing pre-exercise ingestion timing (20). Therefore, the aim of this study was to determine the effects of $NaHCO_3$ supplementation administered in different-sized capsules on blood acid–base responses, GIS, and palatability.

METHOD

Participants

Ten recreationally active male participants, with the following (mean ± SD) characteristics, volunteered for this study: age, 20 ± 2 years; height, 1.8 ± 0.2 m; body mass, 78.0 ± 11.9 kg. All participants undertook regular (≥3 days·wk^{-1}) exercise for at least 30 min per session. Following medical screening, all participants were deemed healthy, free from GI disorders, and not taking any nutritional supplements or prescription medication. The protocol was explained in full, and questions were answered before the participants gave written informed consent to participate in the study. The study was approved by the Departmental Research Ethics Committee.

Study Design

The participants visited the laboratory on three separate occasions after an overnight fast and at the same time of day. The visits were separated by between 24 and 72 h to allow acid–base balance variables to return to normal (21, 22). The participants maintained their habitual diet before experimental testing (23) and refrained from alcohol ingestion and strenuous exercise at least 24 h before each visit. During the initial visit, height (Seca, Germany) and body mass (Holtain, UK) were recorded before the participants consumed 300 mg $NaHCO_3$/kg body mass in gelatine capsules (Bulk Powders™, Colchester, UK). This dose was chosen based on previous findings of improved exercise performance and is a dose widely recognized to be ergogenic within the literature (3, 4, 24, 25). Capsule sizes were administered using a repeated-measures crossover design, following the use of a Latin square to determine trial

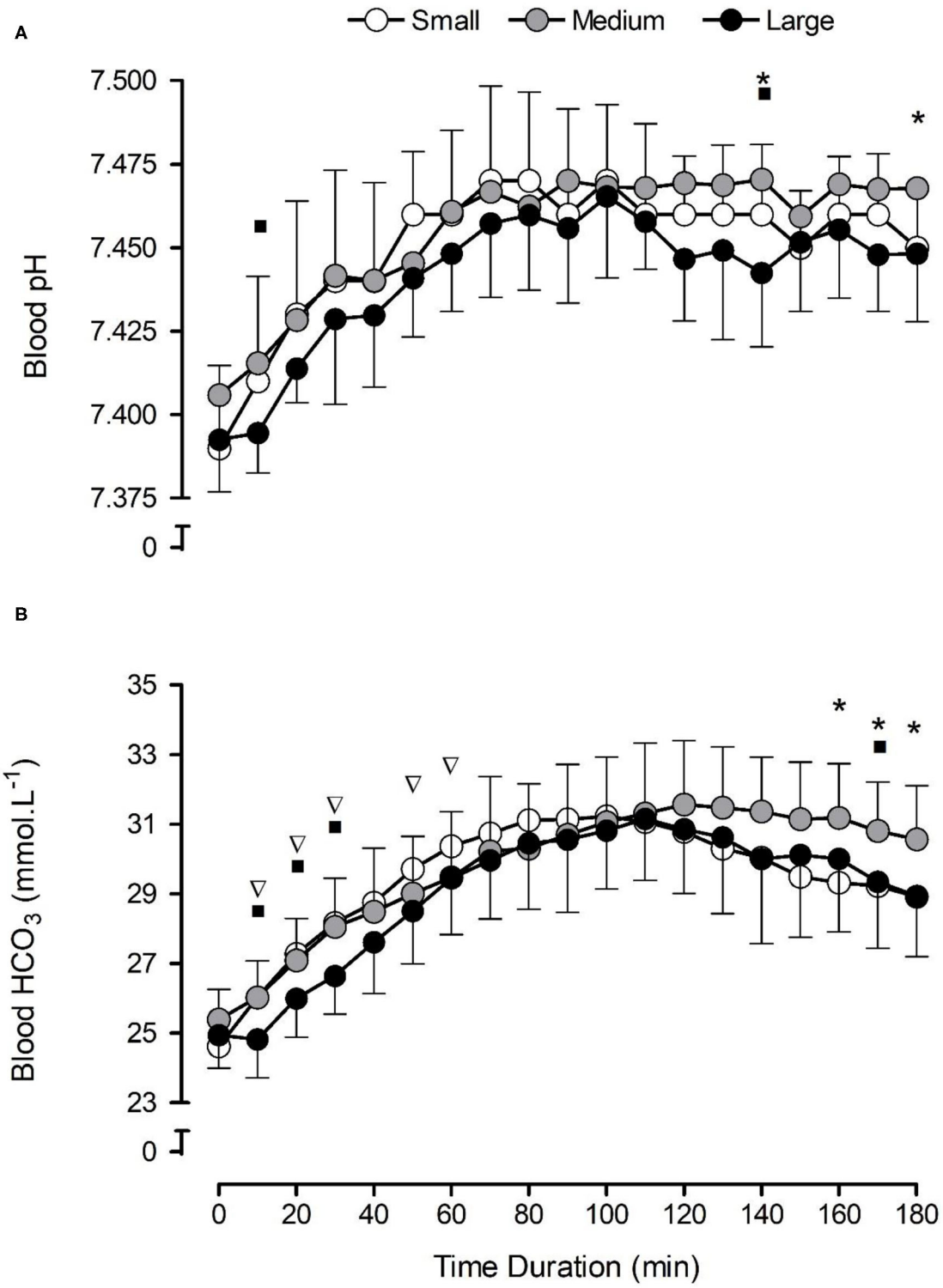

FIGURE 1 | Mean (±SD) temporal blood pH **(A)** and bicarbonate concentration [HCO_3^-] **(B)** responses following the consumption of 0.3 g·kg^{-1} body mass $NaHCO_3$ in small-, medium-, and large-sized capsules. *a condition × time interaction between small and medium capsules, where $p < 0.05$. ∇a condition × time interaction between small and large capsules, where $p < 0.05$. ■a condition × time interaction between medium and large capsules, where $p < 0.05$.

order allocation for participants (26). The three trials used either standard small (size 3), medium (size 0), and large (size 000) capsules. Each capsule contained 0.4, 0.8, and 1.6 g $NaHCO_3$, and the mean number of capsules consumed was 59 ± 9, 29 ± 4, and 15 ± 2, which equated to a total capsule surface area for the bolus of 23.3 ± 3.5, 20.7 ± 3.1, and 16.4 ± 2.5 cm^2 for the small, medium, and large capsule size, respectively. The capsules were consumed with 400 ml of water which was at room temperature (18°C). Capsule palatability was recorded immediately post-ingestion. The participants remained seated for 180 min while blood acid–base responses and GI symptoms were monitored throughout.

Acid–Base Responses

During the experimental protocol, exposure response was established through mapping the time course of blood [HCO_3^-] and potential hydrogen (pH). Fingertip capillary blood procurement was chosen as it is a method widely used in exogenous buffering intervention literature (2, 4, 17, 20, 22) and is a recognized method for blood gas analysis. Capillary blood was drawn pre-ingestion and then post-ingestion every 10 min for 3 h, an established protocol for examining acid–base changes following exogenous buffer ingestion (3, 10, 13). Samples were collected in 100-µl heparin-coated glass capillary tubes (Radiometer Medical Ltd., Denmark) using an aseptic technique and were analyzed immediately using a blood gas analyzer (Radiometer ABL800 BASIC, Denmark). These data were then used to determine the peak in [HCO_3^-] change (C_{max}), the absolute change in [HCO_3^-] (ΔC_{max}), the time to reach C_{max} (T_{max}), the area under the concentration–time curve (AUC), and the time lag (T_{lag}). The T_{lag} was defined as an increase in [HCO_3^-] beyond normal daily variability (13).

Gastrointestinal Symptoms and Palatability

At the same time points, the GI symptoms were measured using a nine-item questionnaire which included stomach cramping, flatulence, nausea, belching, stomach ache, diarrhea, vomiting, bowel urgency, and stomach bloating (27). Each symptom was measured on an 11-point scale, whereby "0 = no symptom" and "10 = severe symptom." Palatability was recorded immediately post-ingestion using a nine-point hedonic scale, where "1 = extremely dislike" and "9 = extremely like" (28).

Statistical Analysis

All data were assessed for normality by the Shapiro–Wilk test and by visual inspection of the normality plots (29). Blood acid–base responses (HCO_3^- and pH) and GI symptoms were analyzed using two-way (condition × time) analysis of variance (ANOVA) with repeated measures. A general linear model ANOVA was used to analyze absolute acid–base values [peak blood [HCO_3^-], time-to-peak blood [HCO_3^-], peak blood pH, time-to-peak blood pH, and area under the curve (AUC)], GI symptoms, and perceived palatability. Two-tailed statistical significance was set at $p < 0.05$. Effect sizes were reported as partial eta-squared (ηp^2) and are described as trivial (<0.20), small (ηp^2 = 0.20–0.49), moderate (ηp^2 = 0.50–0.79), and large (≥0.80), respectively (30).

TABLE 1 | Mean (SD) bicarbonate kinetic variables following the consumption of 0.3 g·kg^{-1} body mass $NaHCO_3$ in small-, medium-, and large-sized capsules.

Variable	Small	Medium	Large
T_{lag} (min)	13 ± 2	22 ± 6	28 ± 4[a]
C_{max} (mmol·L^{-1})	31.7 ± 1.7	32.1 ± 1.5	31.8 ± 1.4
ΔC_{max} (mmol·L^{-1})	7.1 ± 1.1	6.7 ± 1.4	6.8 ± 0.8
T_{max} (min)	94 ± 24[b]	141 ± 27[b]	121 ± 29[b]
AUC (mmol·min·L^{-1})	5,316 ± 256	5,373 ± 264	5,239 ± 263

T_{lag}, lag time; C_{max}, peak bicarbonate concentration; ΔC_{max}, change in peak bicarbonate concentration; T_{max}, time-to-peak bicarbonate concentration; AUC, area under the curve.
[a]Significant difference between the large and small capsules ($p < 0.05$).
[b]Significant difference between all capsules ($p < 0.001$).

RESULTS

Blood Bicarbonate Responses

There were significant increases in blood [HCO_3^-] ($F = 93.2$, $p < 0.001$, $\eta p^2 = 0.91$) in all $NaHCO_3$ conditions compared with pre-consumption values (**Figure 1B**). The capsule size had no significant effect on [HCO_3^-] ($F = 2.3$, $p = 0.151$, $\eta p^2 = 0.21$) post-consumption, although a significant condition × time interaction was observed ($F = 3.3$, $p = 0.014$, $\eta p^2 = 0.27$), suggesting that the large capsules changed [HCO_3^-] more slowly in the initial part of the post-ingestion period, and the medium-sized capsule sustained [HCO_3^-] for a longer time (**Figure 1B**).

Capsule size also had a significant effect on T_{lag} ($F = 3.8$, $p = 0.043$, $\eta p^2 = 0.30$), with significantly longer times in the large-sized (28 ± 4 min) compared with the small-sized (13 ± 2 min) capsules ($p = 0.009$). Similarly, capsule size had a significant effect on T_{max} ($F = 157.6$, $p = 0.000$, $\eta p^2 = 0.94$), with significantly shorter times in the small capsule compared with both the medium-sized ($p = 0.000$) and the large-sized ($p = 0.000$) capsules (**Table 1**). No significant differences were observed for C_{max} ($F = 0.6$, $p = 0.574$, $\eta p^2 = 0.06$), ΔC_{max} ($F = 0.3$ $p = 0.731$, $\eta p^2 = 0.03$), or AUC ($F = 2.1$, $p = 0.148$, $\eta p^2 = 0.19$) between conditions (**Table 1**). There appeared to be a large inter-individual variability in response to capsule ingestion (**Figure 2**).

Blood pH Responses

Blood pH increased in all $NaHCO_3$ conditions ($F = 41.5$, $p < 0.001$, $\eta p^2 = 0.82$) compared with pre-consumption values (**Figure 1A**). Capsule size had a significant effect on blood pH ($F = 3.9$, $p = 0.040$, $\eta p^2 = 0.30$) overall, although no significant condition × time interaction was shown for blood pH ($F = 0.9$, $p = 0.628$, $\eta p^2 = 0.09$; **Figure 1A**). There were no significant differences in either peak blood pH ($F = 1.5$, $p = 0.249$, $\eta p^2 = 0.14$) and time-to-peak blood pH ($F = 1.9$, $p = 0.181$, $\eta p^2 = 0.17$) between conditions.

Gastrointestinal Symptoms and Palatability

Gastrointestinal symptom scores were similar for small-sized (3 ± 3 AU), medium-sized (5 ± 3 AU), and large-sized (3 ± 3 AU) capsules, with no significant difference between symptom scores

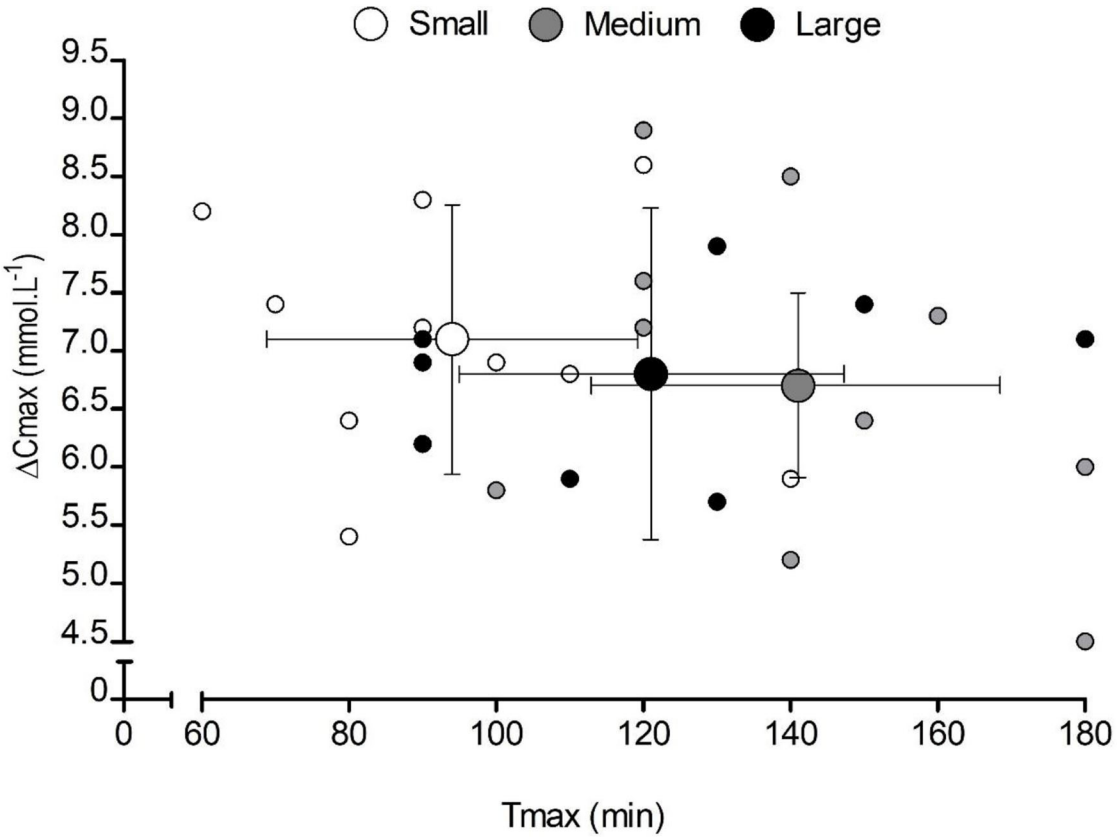

FIGURE 2 | Mean (±SD) and individual peak changes in blood bicarbonate concentration [HCO_3^-] (ΔC_{max}) following the consumption of 0.3 g·kg^{-1} body mass $NaHCO_3$ in small-, medium-, and large-sized capsules. Small markers represent individual responses, and large markers represent the mean data for each capsule condition. The X and Y whiskers represent the SD of the sample in each condition for time-to-peak [HCO_3^-] (T_{max} and ΔC_{max}, respectively).

($F = 1.3$, $p = 0.310$, $\eta p^2 = 0.12$). Similarly, capsule size had no effect on palatability ($F = 0.8$, $p = 0.461$, $\eta p^2 = 0.08$), with similar scores between different capsule sizes (**Figure 3**). The palatability scores ranged from 1–9, 1–9, and 1–7 for small, medium, and large capsule, respectively.

DISCUSSION

This study showed that different capsule sizes led to differences in T_{lag} and T_{max} of blood [HCO_3^-] without affecting the absolute increases in circulating HCO_3^- or AUC of the increases over 180 min. Since T_{lag} (vs. large capsules) and T_{max} was shorter (vs. medium and large capsules) for small capsules, and palatability was similar, albeit also without affecting GI symptoms, this suggests that smaller capsules may be a better form of ingestion for individuals wishing to increase their extracellular buffering capacity more quickly. Those using capsules to administer $NaHCO_3$ should also be cognizant of the trade-off in palatability and participant comfort due to the inverse relationship between capsule size and the number of capsules needed to deliver a potentially ergogenic dose (31). Despite the mean differences in HCO_3^- kinetics when smaller capsules are consumed, we observed considerable individual variability in responses, similar to those previously reported (19, 32–34).

Alternative forms of $NaHCO_3$ ingestion will lead to different pharmacokinetic profiles, with the most common forms in solution or gelatine capsules, with apparently different HCO_3^- kinetics (33). Enterically coated and delayed release forms also lead to different HCO_3^- kinetics compared to gelatine capsules (10, 13, 17). These novel data now show that different sizes of gelatine capsules lead to different blood HCO_3^- kinetics, with quicker increases and time to reach peak values with smaller capsules. Previously, the dissolution rates for individual size 0 and 3 gelatine capsules have been observed to be similar at around 100 s (35). However, in the present study, the large differences between the number of capsules ingested between capsule size conditions results in considerable differences in the total surface area of the ingested substance. Consequently, the greater total surface area of the smaller capsules is likely to liberate their contents quicker. There were no differences between the medium- and large-sized capsules shown here. For those intending to ensure that the start of exercise coincides

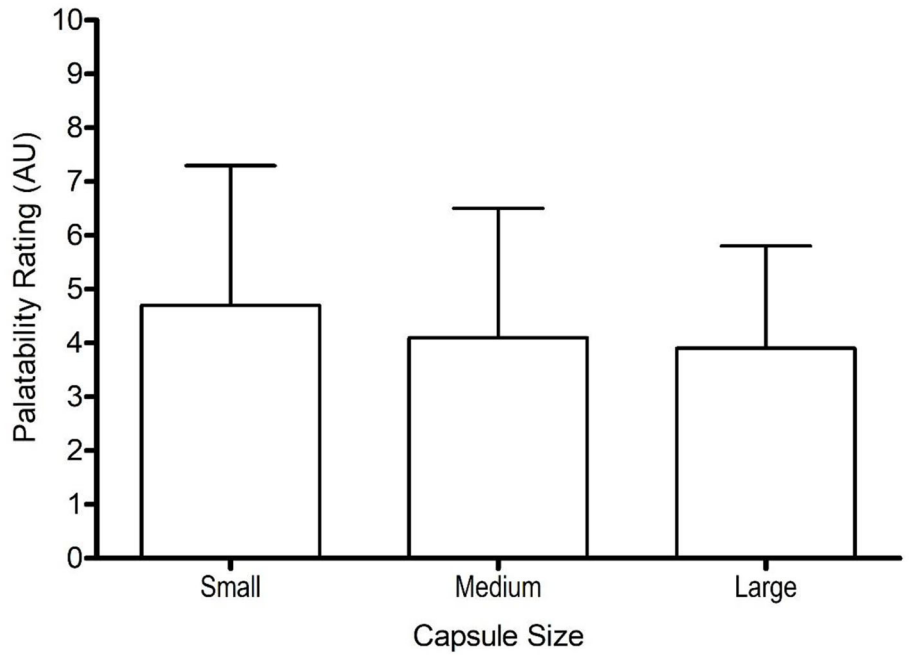

FIGURE 3 | Mean (±SD) palatability scores following the consumption of 0.3 g·kg^{-1} body mass $NaHCO_3$ in small-, medium-, and large-sized capsules.

with C_{max}, these data suggest that individuals could adapt the capsule size in which they ingest $NaHCO_3$ depending on when they can supplement. The present study also standardized the temperature of the fluid ingested with the capsules, but consuming hotter fluids is likely to reduce T_{max}, and colder fluids are likely to increase T_{max} (35). At present, no studies have considered the temperature of the fluid on the pharmokinetics of extracellular buffers such a $NaHCO_3$, but athletes and sports nutrition practitioners should be aware that this is likely to alter the expected time duration at which $NaHCO_3$ should be ingested prior to exercise. It would be of interest to determine whether enterically coated versions of these capsules also lead to different and more favorable HCO_3^- kinetics following ingestion.

Side effects associated with $NaHCO_3$ ingestion include nausea, vomiting, GI discomfort, diarrhea, and headache (4). There have been some suggestions that minimizing neutralization of stomach acids due to the increased $NaHCO_3$ load might lead to reduced GI discomfort and increased circulating HCO_3^- (15). This explains why enterically coated and delayed release forms of $NaHCO_3$ reduce the incidence and severity of GI disturbances compared to gelatine capsules (10, 13, 17). Despite the different HCO_3^- profiles presented here, there were no differences in the side effect symptom scores between the different capsule sizes, suggesting that individuals need not concern themselves with side effects when choosing which size of the gelatine capsule to use for $NaHCO_3$ supplementation. Nonetheless, further work should elucidate whether enterically coated versions of these different capsule sizes can reduce their side effects since discomfort associated with $NaHCO_3$ can be ergolytic to exercise performance (12).

A limitation of this study is that we only analyzed the time course of blood HCO_3^- and pH kinetics following $NaHCO_3$ supplementation in different capsule sizes. It could have been interesting to determine whether different exercise performance responses were shown between the capsules. Nonetheless, it could be hypothesized that similar performance improvements would be shown seen if exercise was performed at TTP since there were no differences between capsule sizes for peak HCO_3^- change, absolute HCO_3^- at peak, and HCO_3^- AUC. It is possible that performance differences might be found should standardized ingestion times be employed prior to exercise since T_{max} was different between capsule sizes. Therefore, it is important to ensure that individual responses to the specific type of capsules that are being used are determined in order to optimize the pre-exercise timing of their ingestion.

In conclusion, small capsule sizes led to quicker T_{lag} and T_{max} of blood [HCO_3^-] compared to medium and large capsules, without affecting absolute increases in circulating HCO_3^- or AUC. The palatability and GI symptoms were similar between all capsule sizes. Individuals could supplement $NaHCO_3$ in smaller capsules if they aim to increase extracellular buffering capacity more quickly.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Department of Sport and Physical Activity Research Ethics Committee, Edge Hill University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IM, SS, and LM designed the study. IM, DT, NL, and NH collected the data. IM, NH, and SS analyzed the data. All the authors contributed to the interpretation of data, writing of the manuscript, have read and approved the final version of the manuscript, and agreed with the final order of presentation of the authors.

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