PERSONALIZED SPORT AND EXERCISE NUTRITION

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PERSONALIZED SPORT AND EXERCISE NUTRITION

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Personalization is a key term when talking about the future of all medical disciplines, including nutrition, and more specifically sport nutrition. The prospect of better tailored and more effective sport nutrition sounds appealing, but the research in personalized sport nutrition is somewhat lacking.

Emerging evidence indicates that sport nutrition supplements and strategies may work in some individuals or under certain conditions, yet not in others. Research on novel ergogenic (= performance-enhancing) dietary approaches is often inconclusive because we fail to understand the environmental and genetic factors impacting the inter-individual responses to their intake and metabolism. These scientific hurdles need to be cleared before we can move to genetic or other screening tests to tailor sport supplement and macro- and micronutrient intake advice.

This Research Topic provided a platform for original data and reviews on novel strategies for personalized sport and exercise nutrition, resulting in a diverse selection of published articles in the area.

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Table of Contents

04 ***Editorial: Personalized Sport and Exercise Nutrition***
Bryan Saunders, Ahmed El-Sohemy and Wim Derave

06 ***Personalized Hydration Strategy Attenuates the Rise in Heart Rate and in Skin Temperature Without Altering Cycling Capacity in the Heat***
Denise de Melo-Marins, Ana Angélica Souza-Silva, Gabriel Lucas Leite da Silva-Santos, Francisco de Assis Freire-Júnior, Jason Kai Wei Lee and Orlando Laitano

12 ***Real-Time Observations of Food and Fluid Timing During a 120 km Ultramarathon***
Floris C. Wardenaar, Daan Hoogervorst, Joline J. Versteegen, Nancy van der Burg, Karin J. Lambrechtse and Coen C. W. G. Bongers

21 ***Pharmacokinetics of ß-Alanine Using Different Dosing Strategies***
Jan Stautemas, Inge Everaert, Filip B. D. Lefevere and Wim Derave

28 ***Effects of Caffeine Ingestion on Anaerobic Capacity in a Single Supramaximal Cycling Test***
Willian E. Miyagi, Romulo C. Bertuzzi, Fabio Y. Nakamura, Rodrigo A. B. de Poli and Alessandro M. Zagatto

37 ***Time to Optimize Supplementation: Modifying Factors Influencing the Individual Responses to Extracellular Buffering Agents***
André B. Heibel, Pedro H. L. Perim, Luana F. Oliveira, Lars R. McNaughton and Bryan Saunders

49 ***Sport Nutrigenomics: Personalized Nutrition for Athletic Performance***
Nanci S. Guest, Justine Horne, Shelley M. Vanderhout and Ahmed El-Sohemy

65 ***A Statistical Framework to Interpret Individual Response to Intervention: Paving the Way for Personalized Nutrition and Exercise Prescription***
Paul A. Swinton, Ben Stephens Hemingway, Bryan Saunders, Bruno Gualano and Eimear Dolan

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EDITORIAL
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Editorial: Personalized Sport and Exercise Nutrition

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Editorial on the Research Topic

Personalized Sport and Exercise Nutrition

Personalized nutrition is an approach that is currently attracting increasing interest in the area of sport and exercise. The prospect of individually tailored, and therefore more effective, sport and exercise nutrition sounds appealing, but research in this area is still in its infancy. To compliment traditional analysis of group mean response, studies are trying to describe the totality of responses by providing individual performance and health data to an intervention which often show large inter-individual variability. Emerging evidence indicates that sport nutrition strategies may work in some individuals or under certain conditions, yet not in others, likely due to a myriad of environmental and genetic factors, highlighting the necessity in providing a more thorough examination of results. The current Research Topic aimed to provide a platform for original data and reviews on novel strategies for personalized sport and exercise nutrition.

The first original article in this Research Topic investigated whether a personalized hydration strategy based upon sweat rate influenced cardiovascular and thermoregulatory responses and exercise capacity in the heat (de Melo-Marins et al.). Participants performed a time-to-exhaustion cycling protocol at 70% of previously determined maximal workload (∼38 min) on three occasions during which they consumed water according to their sweat rate, *ad libitum,* or no fluid. Although the personalized hydration strategy avoided dehydration resulting in lower skin temperature increases and end heart rate, exercise capacity was not different between sessions. This suggests that although an individualized hydration strategy may attenuate thermal strain during exercise in the heat, this does not necessarily translate into performance improvements. Wardenaar et al. used a novel method to monitor the dietary intake of 5 male ultramarathon runners during a 120 km race; researchers used bicycles to follow and film the competitors throughout the race. Large variation in carbohydrate intake was shown between runners, and consumption was well below the 90 $g \cdot h^{-1}$ recommendation for exercise of this duration. Furthermore, only one runner avoided dehydration >2% suggesting that recommendations should be individualized to optimize personal intakes for future races.

Two studies focussed on individual responses to ergogenic aids. Stautemas et al. investigated the pharmacokinetics of beta-alanine in blood following beta-alanine supplementation individualized according to the anthropometric parameters of each participant (10 $mg \cdot kg^{-1}$ body weight) and compared it to a fixed dose of 1,400 mg. Body weight was correlated to the pharmacokinetics and explained a large part of the variation in responses, although the dose individualized to body weight did not reduce this variation in beta-alanine plasma responses. These data demonstrate that there is a heterogenic response to supplementation in an anthropometrically diverse sample suggesting

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further research is warranted to optimize beta-alanine supplementation. Another study showed that acute caffeine (6 mg·kg^{-1} BM) intake increases time-to-exhaustion during a supramaximal effort without any change in estimated anaerobic energy contribution (Miyagi et al.). Individual analysis of the data showed that 10 out of the 14 participants increased exercise capacity above the smallest worthwhile change. Similarly, anaerobic capacity was modified beyond the limits of the smallest worthwhile change in 11 individuals, although the direction of change was highly variable; 4 increased their anaerobic contribution while 7 showed a reduction. Taken together, these data highlight the individual nature of responses to ergogenic supplements and provides scope for further work to elucidate the modifying factors behind these differences.

Two review articles were published in this Research Topic, focusing on the optimization of supplementation with extracellular buffering agents (Heibel et al.) and personalization of nutrition for athletic performance based upon genetics (Guest et al.). Heibel et al. highlighted several factors which may modify the ergogenic effects of buffering supplements such as sodium bicarbonate, sodium citrate and calcium and sodium lactate. These include the timing and dose of supplementation, factors relating to the exercise activity undertaken (e.g., intensity and duration), genetic factors, training status of the individual, and uncomfortable side-effects. The review of Guest et al. outlined a number of genetic factors that may influence absorption, metabolism, uptake, utilization and excretion of nutrients, and food bioactives, which may contribute to physical performance. Specifically, they highlight the current lack of randomized, controlled trials examining the effects of genetic variation on exercise performance in response to nutrients and other food components which may direct future nutritional prescription for athletic performance.

Finally, Swinton et al. provided a statistical framework to support researchers and applied practitioners alike who wish to determine individual responses to a nutritional intervention. This narrative review provides an overview of the fundamental concepts of measurement error and how typical error and confidence intervals can be used to determine uncertainty in measurements. Guidance on how to assess whether meaningful changes have occurred following an intervention is then provided, before discussing the challenges in identifying response or non-response. A modifiable automated spreadsheet is provided free to download to incorporate personal data sets and readers can follow the procedures described within the review to analyse their own responses. Importantly, this statistical framework does not have to be limited to nutritional interventions and can be applied to any dataset wishing to determine an individual's response.

This Research Topic has resulted in some novel articles that have furthered our knowledge in the area of personalized sport and exercise nutrition. We hope that this topic will act as a potent stimulus for further research in this exciting area.

AUTHOR CONTRIBUTIONS

BS, AE-S, and WD are responsible for the writing of the manuscript. All authors approved the final version of the manuscript.

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ORIGINAL RESEARCH
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Personalized Hydration Strategy Attenuates the Rise in Heart Rate and in Skin Temperature Without Altering Cycling Capacity in the Heat

Denise de Melo-Marins[1], Ana Angélica Souza-Silva[1], Gabriel Lucas Leite da Silva-Santos[1], Francisco de Assis Freire-Júnior[1], Jason Kai Wei Lee[2,3] and Orlando Laitano[1]**

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The optimal hydration plan [i.e., drink to thirst, *ad libitum* (ADL), or personalized plan] to be adopted during exercise in recreational athletes has recently been a matter of debate and, due to conflicting results, consensus does not exist. In the present investigation, we tested whether a personalized hydration strategy based on sweat rate would affect cardiovascular and thermoregulatory responses and exercise capacity in the heat. Eleven recreational male cyclists underwent two familiarization cycling sessions in the heat (34°C, 40% RH) where sweat rate was also determined. A fan was used to enhance sweat evaporation. Participants then performed three randomized time-to-exhaustion (TTE) trials in the heat with different hydration strategies: personalized volume (PVO), where water was consumed, based on individual sweat rate, every 10 min; ADL, where free access to water was allowed; and a control (CON) trial with no fluids. Blood osmolality and urine-specific gravity were measured before each trial. Heart rate (HR), rectal, and skin temperatures were monitored throughout trials. Time to exhaustion at 70% of maximal workload was used to define exercise capacity in the heat, which was similar in all trials ($p = 0.801$). Body mass decreased after ADL ($p = 0.008$) and CON ($p < 0.001$) and was maintained in PVO trials ($p = 0.171$). Participants consumed 0 ml in CON, 166 ± 167 ml in ADL, and 1,080 ± 166 ml in PVO trials. The increase in mean body temperature was similar among trials despite a lower increase in skin temperature during PVO trial in comparison with CON (2.1 ± 0.6 vs. 2.9 ± 0.5°C, $p = 0.0038$). HR was lower toward the end of TTE in PVO (162 ± 8 bpm) in comparison with ADL (168 ± 12 bpm) and CON (167 ± 10 bpm), $p < 0.001$. In conclusion, a personalized hydration strategy can reduce HR during a moderate to high intensity exercise session in the heat and halt the increase in skin temperature. Despite these advantages, cycling capacity in the heat remained unchanged.

Keywords: dehydration, thermoregulation, performance, sports, nutrition

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INTRODUCTION

Attenuating fluid loss during exercise in the heat is believed to be an important requirement to maintain performance. The optimal hydration strategy to be prescribed remains a matter of debate (1–4). Research demonstrate that drinking *ad libitum* (ADL) (whenever or in whatever volume desired) and drinking to thirst (relying solely on one's personal sensation of thirst as the only stimulus or guide

drinking) have similar outcomes during exercise (5, 6). A classic paradigm between these two strategies is that blood osmolality is already elevated when thirst mechanisms are activated, which might already be enough to have detrimental impact on endurance performance (7, 8). Personalizing a hydration strategy based on individual's sweat rate appears as an alternative to the ADL and drink to thirst approaches (1, 3), but its influence on physiological and thermoregulatory parameters requires further investigation.

Among the potential advantages of personalizing the hydration strategy are the avoidance of under- and overdrinking, which can hamper performance and health (7). To personalize a hydration strategy requires a pre-assessment of sweat rate, which varies according to exercise intensity and environmental condition and may require athletes and sports nutritionists to periodically monitor changes in body mass in different instances and to make adjustments in the drinking habits accordingly. Likewise, from a performance perspective during races, there is the concern for time lost if one needs to drink more during a critical part of a race/competition. Therefore, having a planned drinking strategy could be beneficial for the race tactic. Despite all these aspects, whether a personalized hydration regimen can be beneficial from a physiological perspective and can translate into positive performance outcomes remains unknown. Research in this field have been inconclusive so far (2, 4). For instance, while one study demonstrated improvements in 16 km running performance with ADL drinking regimen in comparison with a personalized volume (PVO) strategy (4), another study did not find differences between these two strategies (2). Conversely, a recent investigation showed improvements in 5 km simulated uphill cycling performance with a personalized drinking strategy in comparison with ADL (3). The conflicting outcomes from these studies could be due to several factors such as pre-hydration status (i.e., eu vs. hypohydrated), participants' fitness level (i.e., elite vs. recreational), types of drink consumed (i.e., water vs. sports drink), and exercise mode (i.e., running vs. cycling).

Recently, it has been suggested that the success of a given hydration strategy during exercise may also depend on the context of the exercise/sport (duration, intensity, and environment), the characteristics of the individual (fitness and acclimatization status), and the goals of the individual exercising, training, or competing (1). The aim of the present investigation was to test whether personalizing a volume of water based on sweat rate would have beneficial effects on physiological responses and exercise capacity during a moderate duration, high intensity cycling to volitional exhaustion in the heat.

MATERIALS AND METHODS

Participants and Ethical Aspects

Eleven male recreational cyclists (age = 30 ± 7 years; height = 1.77 ± 0.01 m; body mass = 74.7 ± 10.6 kg; body fat = 11.7 ± 0.5%) were recruited. They had at least 3 years of experience with road or mountain bike cycling. Participants visited the laboratory on five separate occasions. They were asked to record their 24 h food intake and then to repeat the same intake before each visit. The study protocol was approved by the University Research Ethics Committee under the number 0015/250614. All participants gave written informed consent after being informed about the risks involved with the study. All procedures conformed to the code of Ethics of the Medical Association (Declaration of Helsinki).

Initial Visit

During the first visit to the laboratory, participants were asked about their normal hydration regimen during exercise to identify any habit that could help with the elaboration of the experimental hydration plan. Thereafter, semi-nude body mass (Marte scale LS200, Brazil) and height were recorded before we performed skinfold assessment (Scientific Skinfold Caliper, Cescorf, Brazil) of the body composition to determine participants' anthropometrical characteristics. Nine skinfolds (biceps, triceps, subscapular, chest, axillar, abdomen, supra iliac, thigh, and calf) were assessed in triplicate by an experienced lab member and percentage body fat was estimated by using the equation proposed by Jackson and Pollock (9). Thereafter, participants undertook a progressive incremental cycling test until exhaustion (CEFISE, model Biotec 200, Brazil). The protocol consisted of an initial workload of 30 W with increments of 30 W/min until exhaustion. The cadence was kept between 60 and 70 rpm, and heart rate (HR) was continuously monitored by a telemetry band (Polar, model S610, Polar Electro, Finland). The test was performed in a warm room with temperature of 34°C and relative humidity of 40% to promote habituation to mechanical, stationary cycling in the heat. Another objective of this visit was to enhance cardiovascular stability and to reduce the interindividual variability in the measurements.

Familiarization and Sweat Rate Assessment

Participants performed two familiarization cycling sessions at 70% of the maximal workload achieved in the previous incremental test (~85% of HR_{max}) for 45 min to minimize learning effects throughout the study and to enhance their heat acclimatization profile. The two sessions were interspersed by at least 2 and at most 4 days to allow appropriate recovery. A fan was positioned at 120 cm of the front of the ergometer with a wind speed of 2.5 m/s to help increase sweat rate due to enhanced convective gradient (10). The session was performed in the heat to mimic the subsequent experimental trials (34°C and 40% relative humidity). Participants' sweat rate was determined during the second familiarization trial based on the change in body mass adjusted by time. This sweat rate was taken into consideration for the calculation of the PVO trial. Percentage (%) dehydration was calculated by multiplying post-exercise body mass by 100 and then dividing it by pre-exercise body mass and subtracting it from 100. Sweat loss was calculated by subtracting the initial from the final body mass in kilograms and then converting to liters, assuming 1 kg corresponds to 1 l. The volume of fluid consumed was added, and urine production was accounted in the equation.

Experimental Hydration Plan

We elaborated a personalized experimental hydration regimen with water based on sweat rate previously assessed in the familiarization session. As participants were not accustomed to large fluid intakes, our goal was to achieve a volume of fluid to offset

80% of the total sweat loss assessed in the previous familiarization session. During the PVO trial, the calculated volume was then administered at a rate of 3.5 ml of water/kg body mass every 10 min.

Experimental Trials

Five days after the last familiarization session, participants performed three experimental trials in the heat. The trials were randomized and interspersed by at least 1 week to avoid the effects of learning and carry over effects of residual fatigue from the previous exercise session, respectively. The exercise intensity and environmental conditions were identical to those previously described in the familiarization sessions. Participants were instructed to self-insert a rectal probe (Physitemp Instruments, New Jersey, NJ, USA) 10 cm past the sphincter to monitor core temperature during each session. Skin temperature sensors (iButton, Maxim Integrated, San Jose, CA, USA) were placed on the chest, upper back, lower back, forearm, thigh, and calf. The sensors were maintained adhered to the skin, despite the presence of profuse sweating, by using a special tape permeable to air and moisture (Fixomull Stretch Tape). Skin temperature was calculated by using the equation proposed by Ramanathan (11). Mean body temperature (MBT) was determined by Hardy and Dubois equation (12): $MBT = 0.8 \times T_{rectal} + 0.2 \times T_{skin}$, where T_{rectal} = rectal temperature and T_{skin} = skin temperature. In the PVO trial, participants were prescribed 3.5 ml/kg every 10 min at minutes 0, 10, 20, and 30. For the ADL trial, bottles containing 800 ml of water were available for participants to drink upon request. Drink temperature for both trials was kept at ~10.5°C to promote consumption (13). In the control trial (CON), participants did not have access to drinks. Cycling time to exhaustion was recorded and used as an indicator of exercise capacity. No verbal encouragement was provided in any trial throughout the study. The test was halted when participants were unable to maintain the cadence. Before exercise, 5 ml blood samples were collected from an antecubital vein while participants were comfortably seated in a semi-recumbent position. Immediately after exercise, participants left the cycle ergometer and sat comfortably again for another 5 ml blood sample. These samples were centrifuged at 2,000 rpm for 10 min used to determine serum osmolality (Microprocessed Osmometer, PZL-1000, Brazil).

Statistical Analysis

A sample size calculation was initially performed with G*Power software taking into consideration previously observed effects size and the requirements of a significance level of $p < 0.05$ from studies determining exercise performance (4, 5). Normality of data distribution was assessed by Shapiro–Wilk test. Repeated measures analysis of variance with Tukey's *post hoc* was performed to compare means among trials. Two-way analysis of variance was employed to compare delta values in temperature and HR. Paired *t*-test was performed to compare the fluid consumption and body mass loss among trials. Data are reported as mean ± SD followed by the degrees of freedom unless otherwise stated. For the purpose of hypothesis testing, the 95% level of confidence was predetermined as the minimum criterion to denote a statistical difference ($p < 0.05$). All data analyses were undertaken using SAS JMP PRO 13 and GraphPad Prism software.

RESULTS

During the incremental test, participants achieved a maximal power output of 372 ± 30 W. Therefore, participants workload during the trials was on average 260 ± 30 W. As described in **Table 1**, participants started all trials with similar hydration status based on urine-specific gravity (USG) and blood osmolality. As observed in **Figure 1**, the time taken to complete all three time-to-exhaustion (TTE) trials was similar regardless of the strategy [37 ± 8 min in the ADL trial, 38 ± 9 min in the PVO trial, and 37 ± 9 min in the CON trial, $p = 0.801$, $F(1.8, 18) = 0.196$] and despite the differences in fluid ingestion and body mass. Cyclists ingested 166 ± 167 ml of fluids in the ADL trial, which was much lower than the volume consumed in the PVO trial, 1,080 ± 166 ml, $p < 0.001$, $t = 20.06$, df = 11. Since we did not provide fluids in the control trial, the fluid consumption was 0. The volume of fluid ingested impacted the hydration status during exercise. In the CON and ADL trials, we observed a reduction in body weight (CON from 75.4 ± 10.3 to 74.5 ± 10.4 kg, $p < 0.001$, $t = 4.831$, df = 11; ADL from 75.4 ± 10.4 kg to 74.6 ± 10.6, $p = 0.008$, $t = 3.171$, df = 11), whereas in the PVO trial body mass did not change (from 75.65 ± 10.18 to 75.52 ± 10.16, $p = 0.171$, $t = 1.462$, df = 11). The changes in body mass reflected the percentage of dehydration which was greater in CON and ADL in comparison with PVO [$p = 0.007$, $F(1.8, 20.5) = 6.38$] as described in **Table 1**. Blood osmolality did not change after the exercise trial regardless of the hydration strategy employed. Although the increase in core temperature and MBT was similar among all trials, we observed a smaller increase in skin temperature in the PVO trial in comparison with CON, $p = 0.038$, $F(2, 15) = 4.70$. Toward the end of the exercise trials, we observed a smaller increase in HR in the PVO trial (162 ± 8 bpm) in comparison with both CON (167 ± 10 bpm) and ADL (168 ± 12 bpm), $p < 0.001$, $F(4, 120) = 229.5$, as demonstrated in **Figure 2**.

TABLE 1 | Urine-specific gravity (USG), serum osmolality, sweat rate, dehydration, and changes in temperatures at the three experimental conditions.

	Control	Ad Libitum	Personalized volume
Pre-exercise USG	1.025 ± 7.6	1.025 ± 7.2	1.026 ± 5.6
Pre-exercise serum osmolality (mOsm/kg)	296 ± 4	294 ± 3	296 ± 4
Post-exercise serum osmolality (mOsm/kg)	297 ± 7	298 ± 6	298 ± 6
Pre-exercise body mass (kg)	75.4 ± 10.3	75.4 ± 10.4	75.6 ± 10.2
Post-exercise body mass (kg)	74.5 ± 10.4	74.6 ± 10.6	75.5 ± 10.2
Sweat rate (l/h)	1.5 ± 0.4	1.4 ± 0.3	1.9 ± 0.5
Dehydration (%)	1.3 ± 0.6	1.0 ± 0.5	0.2 ± 0.4[#]
Δ Core temperature (°C)	0.6 ± 0.3	0.6 ± 0.3	0.5 ± 0.3
Δ Skin temperature (°C)	2.9 ± 0.5	2.6 ± 0.9	2.1 ± 0.9[*]
Δ Mean body temperature (°C)	1.0 ± 0.3	1.0 ± 0.3	0.9 ± 0.4

$p < 0.05$ in comparison with control.
#$p < 0.05$ in comparison with control and ad libitum.
Data are mean ± SD.

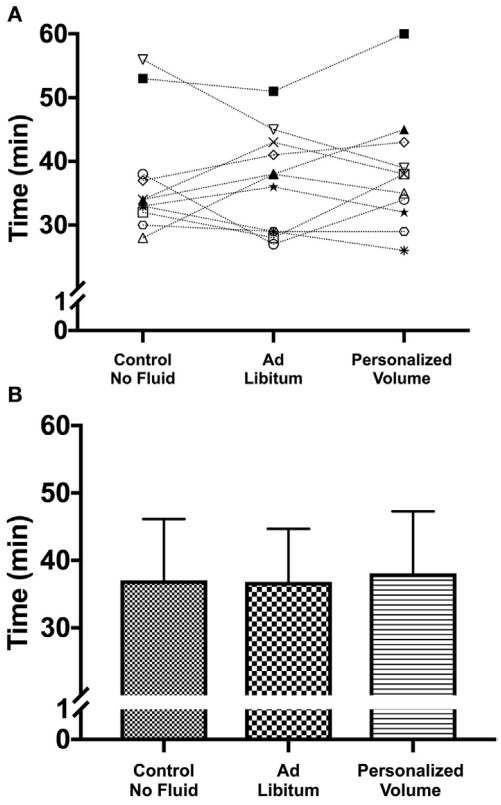

FIGURE 1 | **(A)** Individual performance in the time-to-exhaustion trial in the heat for the three different hydration strategies. Despite the absence of significant differences among hydration strategies it is possible to identify participants whose performance was positively or negatively affected by the personalization of the hydration strategy. **(B)** Mean and SD for each of the experimental trials.

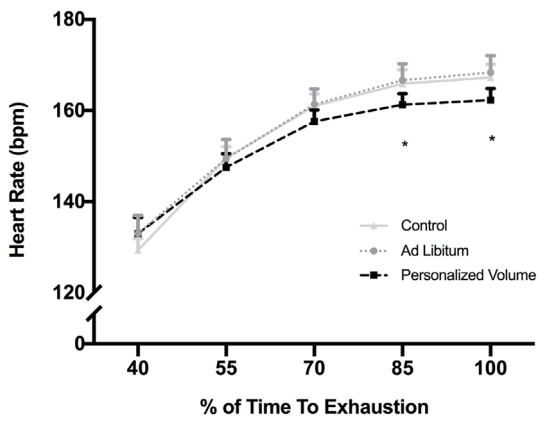

FIGURE 2 | Heart rate response during exercise in the heat for each of the experimental trials. *$p < 0.05$ in comparison with control and *Ad Libitum* trials.

DISCUSSION

The main goal of this study was to determine the impact of a personalized hydration strategy with water on cardiovascular, thermoregulatory and exercise capacity of recreational cyclists while exercising in the heat. Our main finding was that the personalized strategy resulted in lower HR and skin temperature during exercise in the heat and attenuated the degree of dehydration. Importantly, exercise capacity was similar regardless of the hydration strategy employed. These findings add relevant information to previous studies reporting conflicting results on the importance of hydration strategies on physiological outcomes involved in exercise performance in warm environments (2–4, 14).

Hydration guidelines for athletes exercising in the heat encourage consumption of sufficient amounts of fluids during exercise to limit water and salt deficits and also to avoid over drinking (15, 16). Our initial hypothesis was that personalized strategy would have a positive impact in exercise capacity in the heat when compared with ADL fluid intake. Conflicting results have recently been reported in the literature (2–4). For instance, Rollo et al. (4) investigated the effect of ADL versus PVO carbohydrate-electrolyte solution intake on 16 km running performance. Running performance was improved in the ADL trial despite the greater carbohydrate and electrolyte intake in the PVO trial. As they used carbohydrate solution it is difficult to establish a parallel with our model (e.g., hydration with water). Nevertheless, it has been suggested that runners are able to better replace fluid losses in ADL trials in comparison with cyclists (17), which might explain the improved running performance in Rollo et al. study. Conversely, Lopez et al. (2) also investigated the effects of ADL and PVO in euhydrated runners and did not find differences in the time taken to complete 20 km between the two strategies. More recently, Bardis et al. (3) studied the impact of personalized versus ADL hydration strategies on a simulated 5 km high intensity cycling trial. Their findings suggested a performance advantage in the personalized drinking trial. Despite the differences in exercise mode (cycling vs. running), intensity, and type of hydration solution, our results corroborate with Lopez et al. and suggest that performance during exercise in the heat is similar between ADL and PVO hydration strategies. In addition, endurance capacity was not degraded even when no fluid was provided in the context of our experiment.

The combination of exercise, hyperthermia, and dehydration increases cardiovascular strain (18, 19). HR is an important component of Fick equation determining oxygen consumption (VO_2 = [stroke volume × HR] × O_2 a-v difference) and frequently used to monitor cardiovascular response in exercise settings. Our results demonstrate an attenuated HR in the individualized, prescribed volume hydration strategy trial in comparison with ADL intake toward the end of the exercise trial. This occurred along with a lesser degree of dehydration observed in the PVO trial. The hypothesis that personalized strategy would improve endurance capacity was not confirmed in our study. Core temperature was similar regardless of the hydration strategy employed. Even though the total amount of water consumed was significantly higher in the PVO trial, both ADL and no fluid (control) trials elicited significant reductions in body mass that were still within the 2% threshold often considered to negatively influence performance (20) and to affect thermoregulatory responses (21–23). The cutoff of 2% decrease in body mass is believed to be insufficient to impair

performance in acclimated humans (24), such as the cyclists in this study. By using a more intense exercise model, Bardis et al. (3) detected positive influences of a personalized hydration regimen on cycling performance. Their participants dehydrated by 1.8% in the ADL trial. Previous research also reported dehydration levels higher than 3% with no performance impairment (23, 25). Further studies are required to confirm the hypothesis that prescribing a fixed volume of fluids based on individual sweating rates would be beneficial to humans experiencing dehydration levels >2% of body mass loss. Nevertheless, our findings seem to be in alignment with a recent statement that ADL drinking would be adequate during exercises with durations ranging from 60 to 90 min; whereas planned drinking would be recommended for longer duration activities of >90 min, particularly in the heat where performance is a concern (1).

The total volume of fluid consumed during the PVO trial was fivefold higher in comparison with the ADL trial. ADL hydration strategies are usually associated with low fluid intake (4), mainly in euhydrated participants (26). Bardis et al. reported an ADL fluid consumption of ~0.7 l in their cohort (3). In our study, particularly in the ADL trial, participants did not drink enough to offset sweat loss, which might indicate euhydration. Our participants were used to exercise in the heat and not to drink large volumes of fluid. Therefore, it is possible to conjecture that psychophysiological factors could explain the drinking patterns we observed in this study. Another interesting finding of our study was the blunted increase in skin temperature when PVO was used as hydration strategy. Skin temperature is an important variable regulating heat exchange *via* sweat evaporation (27). Even though it did not reflect in performance improvements, maintaining a lower skin temperature can be advantageous during exercise in the heat to allow heat transfer from core to the skin (28). Further studies are required to explore the mechanisms by which a lower skin temperature is observed when a PVO is adopted in comparison with ADL and CON.

A post-exercise urine sample to determine urine osmolality could add an additional marker of participants' hydration status in addition to our current measurement of blood osmolality. It is, however, well known that urine production after exercise is directly related to renal blood flow, which is known to be markedly reduced during exercise, especially in the heat where there is an increase in anti-diuretic endogenous hormones (i.e., vasopressin) (29). While a few participants provided a urine sample after exercise in the PVO trial, none was able to provide a urine sample after CON and ADL trials and therefore making the measurement of USG and osmolality not possible. Importantly, post-exercise urine osmolality has questionable sensitivity to detect hydration status (30). It is possible that the relatively short duration (~45 min) of the exercise trial coupled with the modest dehydration (~1% decrease in body mass) achieved in the ADL and CON trials could have accounted for the lack of differences among all hydration strategies in this study. Nevertheless, it is our understanding that the current results add to an already existing body of literature on the pros and cons of providing a PVO of fluids based on sweat rate to exercising humans (2, 4). It is worth to highlight that participants in our study were naturally acclimated to heat as they lived and trained in a tropical climate region. Exercise in the heat promotes long-term adaptations in sweat rate, skin blood flow, plasma volume expansion, and a greater cardiovascular stability (31, 32), which could have influenced the results herein described. Importantly, we only studied the responses of men. Whether the results of this study hold true for women requires further investigation as factors such as menstrual cycle are known to play a role in fluid balance and thermoregulation during exercise in the heat (33).

In conclusion, our study demonstrates that PVO hydration strategy was effective in reducing the cardiovascular stress and the increase in skin temperature imposed by a cycling session to exhaustion in the heat. Despite the lower cardiovascular and thermoregulatory stresses, the exercise capacity was similar regardless of the hydration strategy employed.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Federal University of Vale do São Francisco Research Ethics Committee. The protocol was approved by the #0015/250614. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

OL and JL conceived and designed the experiment. DM-M, AS-S, FF-J, and GS-S performed the experiment. DM-M, OL, and JL analyzed and interpreted the results. DM-M, AS-S, FF-J, GS-S, JL, and OL wrote and approved the final version of the manuscript.

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Real-Time Observations of Food and Fluid Timing During a 120 km Ultramarathon

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The aim of the present case study was to use real-time observations to investigate ultramarathon runners' timing of food and fluid intake per 15 km and per hour, and total bodyweight loss due to dehydration. The study included 5 male ultramarathon runners observed during a 120 km race. The research team members followed on a bicycle and continuously observed their dietary intake using action cameras. Hourly carbohydrate intake ranged between 22.1 and 62.6 g/h, and fluid intake varied between 260 and 603 mL/h. These numbers remained relatively stable over the course of the ultra-endurance marathon. Runners consumed food and fluid on average 3–6 times per 15 km. Runners achieved a higher total carbohydrate consumption in the second half of the race ($p = 0.043$), but no higher fluid intake ($p = 0.08$). Energy gels contributed the most to the total average carbohydrate intake (40.2 ± 25.7%). Post-race weight was 3.6 ± 2.3% (range 0.3–5.7%) lower than pre-race weight, revealing a non-significant ($p = 0.08$) but practical relevant difference. In conclusion, runners were able to maintain a constant timing of food and fluid intake during competition but adjusted their food choices in the second half of the race. The large variation in fluid and carbohydrate intake indicate that recommendations need to be individualized to further optimize personal intakes.

Keywords: running, sports nutrition, recommendations, supplements, sweat rate, fluid balance

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INTRODUCTION

An ultramarathon is any footrace longer than the traditional marathon length of 42.195 km. Ultra-endurance performance is highly dependent on the intake of carbohydrates during the race. In addition to muscle glycogen stores, up to 60–90 g of exogenous carbohydrates per hour are advised to maintain relatively high exercise intensity levels during ultra-endurance performance (1–5). Carbohydrate oxidation in recreational well-trained, non-fat adapted runners has shown to be the dominant energy source (≥70% of the total energy expenditure) (6, 7). Previous studies investigating nutrient intake in ultra-endurance events reported a wide range of carbohydrate intake (23–71 g/h), which was often lower than the recommended intake of 60–90 g carbohydrates per hour (8–19).

Besides the need to consume sufficient carbohydrates, ultramarathon runners need to prevent excessive dehydration (>2% body weight loss from water deficit) particularly in prolonged exercise

lasting greater than 3 h (20). Previously reported fluid intake in ultra-runners was 354–765 mL/h (8, 10, 11, 13–19, 21). In some of these studies, fluid balance was measured by indication of pre and post-race body weight, but actual sweat rate estimation was not reported. In other sports, with a shorter duration (i.e., waterpolo, netball, swimming, rowing, basketball, soccer, American football, tennis, and running), sweat rate estimates range from ∼0.3 to ∼1.8 L/h (20). Overall, it can be debated if fluid intake during previous ultramarathons was optimal or could be improved.

Several factors might limit the carbohydrate and fluid intake over the course of the ultra-endurance running event. These factors may include, but are not limited to, environmental conditions (i.e., temperature, humidity) (14), exertion as a result of competition, pacing strategy, restricted feeding options during competition (22), the aid stations design (i.e., placing, frequency, and product range), and gastro-intestinal (GI) distress (14). Many ultra-endurance runners experience gastro intestinal complaints during an ultra-endurance competition, which may complicate the regular ingestion of nutrients and fluid during the race (17, 23). GI distress can be divided by upper and lower GI complaints. Both the upper and lower GI complaints likely have different mechanisms, but they all limit feeding as a result of discomfort (24). Interestingly, we previously demonstrated that a large set of GI complaints (i.e., heartburn, belching, vomiting, intestinal cramp, urge to defecate, side ache, loss of stool, and diarrhea) are negatively correlated with the intake of energy, carbohydrate, and fluid (17).

Food and nutrient intake during ultra-marathons has often been self-reported using a questionnaire or recalls (15, 18, 19, 25). However, recalling all foods and drinks ingested during a daylong ultra-endurance event is difficult, thereby hampering the validity of the results. Moreover, most studies only reported total food and nutrient intake, rather than the timing of food, and nutrient intake over the course of the ultra-endurance event.

Although sufficient intakes of carbohydrates and fluid are essential for ultra-endurance performance, actual intakes are the result of the timing and amounts of food and fluid consumed during competition. So far, only one $n = 1$ female case study by Moran et al. (13) reported timing of food and fluid during an ultramarathon. Because feeding strategies differ between athletes, more observations of timing of food and fluid intake are needed. Therefore, the aim of the present study was to use real-time observations to investigate whether ultramarathon runners were able to maintain constant intakes of carbohydrates and fluid to meet recommendations over the course of a 120 km ultramarathon.

MATERIALS AND METHODS

Study Design

This cross-sectional study was designed to provide descriptive information using real-time observation on food and fluid intake, hydration status, and GI complaints in ultra-endurance athletes in April, 2017, in the Netherlands. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethical Advisory Board of the HAN University of Applied Sciences (EACO 63.03/17). Written informed consent was obtained from all runners.

Study Population

Runners registered for the 120 km distance of the Zestig van Texel ("Sixty of Texel") event in the Netherlands. All runners included in this study completed a short web-based questionnaire about their personal characteristics, general health and medical condition, and running history. Nine healthy male runners without injuries, from 18 to 55 years old (47 ± 6 years), participated. Each of the runners had previously completed at least 10 (ultra)marathons during their running career. Two of the runners said to use a carbohydrate rich diet in the days before the race, one of the runners said to focus mainly on extra fluid intake and five runners did not change their dietary intake in preparation for the race day. One runner said to follow a high fluid and carbohydrate rich diet in the days before the race.

Procedures

All selected runners received extensive information at least 5 days before the race. An interview was scheduled for the day before the race with all included runners. During the interview, the food and beverage labeling and product registration was explained. The organization of this ultramarathon allowed runners to be cared for by a personal cyclist that could hand out beverage and food products throughout the whole race. The most important reason for this is runners of the 120 km cover two sections of 60 km, but during the first 60 km no aids stations were available. Runners brought their own products and were allowed to have their own nutrition strategy. They labeled their own products with a unique code that was provided to them, and a research team member double-checked whether this was done correctly. Next, all products were weighed, including the original wrapping when relevant. Pictures were taken of all foods and beverages, including labels and ingredient declarations.

On the day of the race, body weight and height (Cescorf stadiometer, Porto Alegre, Brazil) were measured (without shoes in light racing clothes) 1 h before the start (5.35 a.m.). Runners were asked to empty their bladder before measuring body weight. Thereafter, all food and fluid intake was observed and urine excretion collected until completing the ultramarathon. During the race, the runners were accompanied by a research team member on a bicycle for continuous observation of nutritional intake and urine excretion. The member recorded the time for each 15 km milestone. Runners reported GI complaints, as well as their rating of perceived exertion (RPE) using a 6–20 categorical Borg scale. Directly after completing the race, body weight, total urine excretion, and the weight difference of all consumed products were measured.

Dietary Intake

Observation of Intake

Action cameras were attached to the bicycles (SJCAM, SJ4000, Shenzhen, China) to record all food and fluid consumptions. During the registration, the observant reaffirmed with the runner, on camera, all consumed products and beverages, each 15 km milestone time (hh:mm:ss), RPE and GI complaints.

Products

Food products and fluids, including wrapping or wrapping only, were measured on a digital scale (Cresta, CKS750, Amsterdam, The Netherlands) before and after the race with 0.1-g accuracy. The difference between measurements was calculated to obtain the actual consumed amount of each product (g). In addition, all foods were categorized as: sports drink (isotonic and hypertonic formulas), gels, cola, chocolate milk, water, other fluid (all other drinks consumed), other solid (all other products consumed), and bars.

Nutrients

Based on the total product weight and ingredient declarations, the total consumed amount of energy (kcal); grams (g) of carbohydrate, protein, fiber and fat; and milliliters of fluid (mL) were calculated.

Fluid Balance

Body Weight Difference

Body weight (Seca scale S760 mechanical, Hamburg, Germany) was measured pre- and post-race. Furthermore, the absolute and relative body weight loss (difference between pre- and post-race) was calculated, in which the relative body mass is presented as dehydration level (%).

Urine Collection

The runners were instructed to urinate into a specialized collecting bag (Roadbag, KETs GmbH, Köln, Germany) from 60 min pre-race until directly after the race to determine the total urine excretion. The urine bags were provided and collected as needed at all points during the race. However, due to the inability to cycle at the beach sections of the race, it was not possible to collect urine (24.5 km total). During the race, only one urine excretion that took place on the beach section was not collected. For this runner, an estimated amount of 125 ml was added to his total collected urine excretions to calculate total urine loss. The urine bags were weighted after the race with a 0.1 g accuracy (PT 1400, Sartorius AG, Göttingen, Germany).

Sweat Rate

The estimated sweat rate was calculated as total weight lost, corrected for all consumed foods and beverages and collected urine during the race (as: weight loss (kg) + Fluid and beverages consumed in kg–total urine loss in kg). As respiratory water losses and the production of metabolic water as a result of cellular metabolism are approximately equal, these were not included in this equation (20).

Measurements per 15 Km

RPE and Split Time

Within 200 m before each 15 km milestone, runners were asked to appoint their rating perceived exertion (RPE). Before the race, they were familiarized with a 6–20 categorical Borg scale, in which 6 was very light and 20 very hard. Furthermore, at every 15 km milestone, the split time was noted, which was used to examine the pacing strategy of the runners.

GI Distress

The runners were instructed to report GI complaints using a questionnaire at every 15 km milestone. The GI complaints were assessed using a previously described pre-specified list of complaints, which was also used prior to and directly after finishing the race. After the race, runners were asked to fill out, within 24 h, a previously described questionnaire (17) in which runners identified again all GI complaints they experienced over the race. In both cases, the runners were instructed to rank the specific identified GI complaints on a nine-point scale ("no problem at all" to "the worst it has ever been"). If a complaint had a score >4, it was considered serious as described previously by Pfeiffer et al. (14).

Data Analysis

All calculations were performed in Excel (2016) and SPSS (IBM SPSS Statistics, version 23). Characteristics of runners—i.e., finish time (hh:mm), speed (km/h), height (cm), weight (kg), consumption moments, combined food and fluid intake (kg), urine excretion (L), sweat rate (L), and absolute and relative body weight loss (kg and %)—were reported as individual and group data (mean±sd). Total consumed food and fluid per runner and for the group (mean±sd) were expressed as total energy (kcal), carbohydrates, protein, fiber (in g/h), and fluid (mL/h). The average CHO intake per hour was expressed per food group for each individual. The total amount of consumed foods per category (g) were expressed per 15 km milestone, as well as nutrient intake (CHO, PRO, fluid, fiber, fat), pacing (min/km), RPE, consumption moments, and urine excretion (g). GI complaints during the race and GI complaints based on the questionnaire were reported as descriptive (incidence and score).

Difference between pre-race and post-race weight and difference between running speed and average nutrient intake per 15 km or per hour during the first and the second part of the race were tested based on the Wilcoxon Signed Rank test, with the level of significance set at $p \leq 0.05$.

RESULTS

Exercise and Environmental Conditions

Complete datasets were collected for only 5 of the subjects, as 3 runners withdrew from the race at the 60 km turning point and 1 runner dropped out earlier because of an injury. Runners completed the 120 km distance with an average speed of 9.9 ± 1.5 km/hour, as shown in **Table 1**. Running speed during the first part of the race was higher than in the second part, which resulted in a faster first 60 km compared to the second 60 km (05:32 ± 00:30 h vs. 06:47 ± 01:01 h, respectively, $p = 0.043$). The RPE at start was 6.2 ± 0.4 vs. a RPE at finish of 16.2 ± 1.9). The average temperature on the day of the race was 7.0°C (min: 4.3°C and max: 9.6°C). Humidity was 67% with 1.6 h of rain and 11.1 h of sunshine between sunrise and sunset. The average wind speed was 5.0 m/s (measured at weather station De Kooy, Den Helder).

Food and Carbohydrate Intake

Total energy and nutrient intake varied substantially between the five runners. Each runner consumed products from 3 to

5 of the previously defined food categories (**Figure 1**). Total energy intake during the race varied between 1,435 and 3,777 kcal. The combined total food and fluid intake was 5.4 ± 1.7 kg with a total of 0.2 ± 0.2 kg of solid foods. Carbohydrates were mainly delivered as part of fluids. Total mean fluid-based carbohydrate intake was 440 ± 193 vs. 91 ± 83 g of carbohydrates delivered by solid foods. Energy gels contributed the most to the total carbohydrate intake (43.6 ± 19.1 g/h). Individual carbohydrate intake ranged between 22.1–62.6 g/h, and fluid intake (421±127 mL/h) varied between 260–603 mL/h. As shown in **Table 2**, product choices in the first 60 km of the race were very consistent (i.e., consumption of energy gel, water, and sport drink). During the second part of the race other types of foods were introduced to the pattern. Although water and sports drinks remained the most important energy and fluid sources during the second part, chocolate milk and cola also contributed. Overall a less structured pattern was seen in the second part of the race in comparison to the first part of the race.

Fluid Intake and Estimated Sweat Rate

Post-race body weight was lower in 4 out of 5 runners (**Table 1**). Total fluid intake, based on the consumption of liquids, was 5.1 ± 1.6 L, which was lower than a total estimated sweat rate of 6.6 ± 2.3 L. The average urine excretion was 0.8 ± 0.5 L. Furthermore, the level of dehydration of the runners after completing the race was 3.6 ± 2.3% (range 0.3–5.7%), which was not significant ($p = 0.08$).

Timing of Food and Fluid Intake

Table 3 shows that runners had 3–6 consumption moments per 15 km during the race. The highest observed number of consumption moments during the race was observed in the second part of the race (after completing 75 km). In this 30 km period, between 75–90 and 90–105 km, the total number of consumption moments was 6.2 ± 1.1 and 5.6 ± 3.5 consumption, respectively. The larger total intake resulted also in the highest intakes of carbohydrates (90.6 ± 38.2 and 79.0 ± 43.9 g/h) and fluid (950 ± 429 and 907 ± 394 mL/h) during the race.

TABLE 1 | Characteristics of ultramarathon runners during 120 km race ($n = 5$).

Runner	Finish time (hh:mm)	Average speed (km/h)	Height (cm)	Pre-race weight (Kg)	Post-race weight (Kg)	Consumption moments	Food/fluid intake (Kg)	Urine excretion (L)	Fluid loss, sweat rate (L)	Absolute body weight loss (Kg)	Level of dehydration (%)
1	09:50	12.2	184	71.5	69.0	48	4.7	1.1	6.0	2.5	3.5
2	11:26	10.5	178	65.5	61.8	36	4.2	0.5	7.5	3.8	5.7
3	13:08	9.1	185	78.0	74.5	48	7.9	0.3	10.1	3.5	4.5
4	13:20	9.0	187	75.0	75.2	25	5.3	0.6	4.6	−0.2	0.3
5	13:49	8.7	178	65.8	62.7	31	3.8	1.4	4.9	3.1	4.6
Mean ± *SD*	12:19 ± 01:29	9.9 ± 1.5	182 ± 4	71.2 ± 5.5	68.6 ± 6.3	37.6 ± 10.3	5.4 ± 1.7	0.8 ± 0.5	6.6 ± 2.3	2.5 ± 1.6*	3.6 ± 2.3

*****No significant difference between pre-race and post-race weight based on Wilcoxon Signed Rank Test, p = 0.08.*

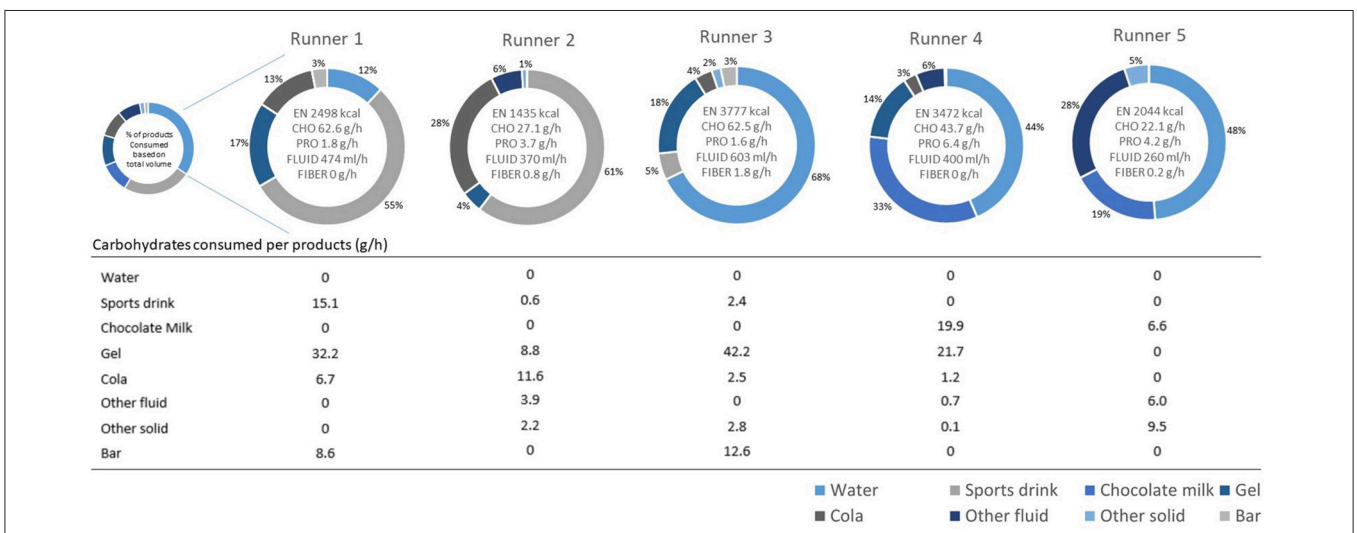

FIGURE 1 | Total energy and nutrient intake per hour, percentage (%) volume of total consumed food groups, and carbohydrate intake (CHO) per food group in g/h per runner.

TABLE 2 | Consumed products per 15 km (volume in g) and most used products on group level (person count).

	0–15 km	15–30 km	30–45 km	45–60 km	60–75 km	75–90 km	90–105 km	105-–120 km
Runner 1	Sport drink (518 g) Sport bar (43 g) Gel (77 g)	Sport drink (259 g) Gel (141 g) Sport bar (22 g)	Sport drink (389 g) Gel (72 g) Sport bar (43 g)	Sport drink (259 g) Gel (63 g) Sport bar (22 g)	Sport drink (289 g) Gel (77 g) Sport bar (22 g)	Sport drink (259 g) Gel (148 g) Water (138 g)	Cola (450 g) Sport drink (389 g) Water (330 g) Gel (122 g)	Cola (150 g) Gel (131 g) Sport drink (130 g) Water (100 g)
Runner 2	Sport drink (539 g) Gel (78 g)	Sport drink (606 g) Gel (109 g)	Sport drink (439 g) Gel (132 g)	Sport drink (414 g) Gel (138 g)	Sport drink (138 g)	Sport drink (276 g) Cola (150 g) Other solid (47 g) Sweets (11 g)	Cola (300 g) Sport drink (230 g)	Cola (750 g)
Runner 3	Gel (355 g) Water (230 g)	Water (517 g) Gel (174 g) Other solid (67 g)	Water (430 g) Gel (267 g)	Water (602 g) Other solid (54 g)	Water (1,016 g) Gel (163 g) Other solid (109 g)	Water (844 g) Gel (347 g) Sport drink (150 g) Fruit (83 g)	Water (974 g) Gel (153 g) Other solid (62 g) Fruit (17 g)	Water (946 g) Sport drink (300 g) Cola (300 g) Fruit (33 g)
Runner 4	Water (446 g) Gel (160 g)	Water (408 g) Gel (160 g)	Water (676 g) Gel (331 g)	Gel (108 g) Water (78 g)	Choc. milk (418 g)	Choc. milk (361 g) Water (349 g) Cola (150 g) Fruit (20 g)	Choc. milk (779 g) Other fluid (330 g) Water (182 g)	Choc. milk (256 g) Water (229 g)
Runner 5	Water (120 g)	Water (360 g) Other solid (28 g)	Water (250 g) Other solid (41 g)	Other fluid (256 g) Water (250 g) Other solid (70 g)	Choc. milk (245 g)	Water (983 g) Other fluid (256 g) Choc. milk (248 g) Other solid (70 g)	Other fluid (337 g)	Choc. milk (261 g) Other fluid (254 g)
#1 used products	Gel (4)	Gel (4)	Gel (4)	Water (3)	Choc. milk (2) Sport drink (2)	Water (4)	Water (3)	Cola (3) Water (3)
#2 used products	Water (3)	Water (3)	Water (3)	Gel (3)	Gel (2)	Sport drink (3)	Cola (2) Sport drink (2) Gel (2) Other fluid (2)	Sport drink (2) Choc. milk (2)
#3 used products	Sport drink (2)	Sport drink (2) Other solid (2)	Sport drink (2)	Sport drink (2) Other solid (2)		Choc milk (2) Cola (2) Gel (2) Fruit (2) Other solid (2)		

TABLE 3 | Surface, Pacing, rate of perceived exertion, eating moments, fluid excretion, and nutrient intake per 15 km ($n = 5$).

	ASPHALT		GRAVEL BEACH ASPHALT BEACH	ASPHALT BEACH	SOIL BEACH GRAVEL	ASPHALT DUNE ROADS	ASPHALT
	0–15 km	**15–30 km**	**30–45 km**	**45–60 km**	**60–75 km**	**75–90 km**	**90–105 km**	**105–120 km**
Pacing (min/km)	5 : 41 ± 0 : 32	5 : 21 ± 0 : 17	5 : 34 ± 0 : 32	5 : 38 ± 0 : 44	6 : 45 ± 1 : 24	6 : 57 ± 0 : 47	6 : 12 ± 0 : 46	7 : 08 ± 1 : 47
RPE	8.4 ± 2.1	9.6 ± 1.7	11 ± 2	12.4 ± 3.1	14.6 ± 2.7	15.2 ± 2.2	15 ± 3.8	16.2 ± 1.9
Eating moments	4.0 ± 2.1	4.6 ± 0.9	5.6 ± 1.8	3.8 ± 0.8	3.0 ± 2.8	6.2 ± 1.1	5.6 ± 3.5	4.8 ± 2.0
Urine excretion (mL)	144 ± 127	276 ± 218	59.0 ± 76.2	18.4 ± 36.8	25.0 ± 50.0	87.2 ± 149	69.9 ± 99.1	112 ± 92.3
CHO (g)	58.5 ± 48.8	60.5 ± 32.9	73.0 ± 38.1	48.2 ± 15.3	58.1 ± 42.7	90.6 ± 38.2	79.0 ± 43.9	61.2 ± 16.9
PRO (g)	2.1 ± 3.0	3.1 ± 2.9	2.5 ± 2.5	4.7 ± 3.8	8.3 ± 7.5	9.2 ± 7.3	10.5 ± 14.3	5.3 ± 6.0
Fluid (mL)	505 ± 193	551 ± 141	598 ± 247	430 ± 151	489 ± 374	950 ± 429	907 ± 394	766 ± 414
Fiber (g)	0.2 ± 0.4	1.9 ± 2.2	0.8 ± 1.0	0.6 ± 0.9	3.7 ± 3.8	4.6 ± 0.7	1.1 ± 2.1	0.1 ± 0.3
FAT (g)	0.1 ± 0.2	0.5 ± 0.5	0.4 ± 0.7	4.6 ± 5.5	6.5 ± 6.7	7.1 ± 8.2	7.8 ± 12.3	4.4 ± 5.4

Surface is shown from the start (left side of the figure) until the finish after 120 km (right side of the figure), each column within the table represents a 15 km distance. All data represents mean values ± SD per 15 km.

The pattern differed between the first and the second 60 km of the race. The absolute carbohydrate intake per 15 km was lower for the first 60 km of the race than the second part (60.1 ± 33.8 vs. 72.2 ± 35.4 g/15 km, $p = 0.04$). No difference for carbohydrate intake was seen when intake was divided in two equal time periods based on finish time ($p = 0.69$), as average carbohydrate intake was 46.9 ± 17.0 vs. 46.5 ± 14.1 g/h. No differences were detected for fluid intake between the first and second 60 km of the race (521 ± 183 vs. 778 ± 403 mL/15 km, $p = 0.08$). Likewise, no difference was seen for fluid intake between the first and second half of the race based on finish time (379 ± 89.0 vs. 461 ± 161 mL/h, $p = 0.35$).

Gastro Intestinal Complaints

Runners reported 1–2 GI complaints during the race, but the type and frequency of complaints as well as the severity (score 3–8) of these complaints varied between runners (**Table 4**). Interestingly, during the race, runners reported a substantially lower amount of experienced complaints in comparison to the self-reported complaints in the post-race questionnaire (1–2 reported during competition vs. 3–8 self-reported after competition per person). Based on this questionnaire, all runners reported both upper and lower GI complaints. In the post-exercise questionnaire, runners reported reflux ($n = 3$), nausea ($n = 2$), belching ($n = 2$), and bloating ($n = 3$), which all might result in perceived discomfort. Finally, three out of five runners also reported an urge to urinate during the race, based on the questionnaire.

DISCUSSION

The aim of the present study was to use real-time observations to investigate whether ultramarathon runners were able to maintain constant intakes of carbohydrates and fluid to meet recommendations over the course of a 120 km ultramarathon. Despite the fact that all runners experienced GI complaints during the event, the intake of carbohydrate and fluid per hour remained relatively stable during the race. The unique setting of this race, allowing the runners to have continuous feedings opportunities delivered by their accompanying cyclists, showed only small variations in CHO intake between runners for each 15 km covered. On the contrary runners were not able to constantly consume a high amount of carbohydrates and fluid intake was on average much lower than the estimated sweat rate.

Overall carbohydrate and fluid intake in the present study was lower than 2013 reports from other Dutch ultramarathon runners, where the observed intake was >30 g of carbohydrate and >350 mL of fluid per hour (17). On the other hand the reported average CHO and fluid intake of the present study fits within the range of earlier reports, reporting a carbohydrate intake 37.0–67.2 g/h and a fluid intake of 354–765 mL/h (10–13, 16, 17). These reports used observation or recall during the race instead of post-race questionnaires during different ultramarathons.

To our knowledge this is the first study in 20 years capturing urine excretion during competition (26). During the present data collection, urine excretion was, on average, 11% of the total weight lost vs. 4.6% urine loss reported in a multi-event study by Rogers et al. (26). The focus of the multi-event study was to assess fluid balance, including urine excretion, at a higher temperature than the current study (7 vs. 28°C). This temperature difference might explain a part of the disparity in the percentage of urine loss between studies. Also, the type of fluids consumed and amount of carbohydrates ingested may have influenced fluid retention. Despite individual differences for hydration potential, we calculated that, on average, 15.7% of total fluid intake was lost as urine. A recent randomized trial showed a difference between beverage hydration indexes of commonly used fluids. The article showed that drinks with the highest macronutrient and electrolyte contents—like oral rehydration salts (ORS), orange juice, full fat milk, and skim milk—were the most effective in maintaining fluid balance at rest (27). It is uncertain if the results of this method also apply to fluid retention potential during exercise. Given that most of the runners already consumed highly concentrated gels and bars, and three of them consumed chocolate milk during the race, urine excretion in the current study still equalled 30% of the exercise-induced dehydration. Limiting urine excretion by better fluid retention could therefore potentially help these athletes to

TABLE 4 | Gastro intestinal complaints assessed during the race and based on a post-race questionnaire ($n = 5$).

	During the race (assessed each 15 km)			Post-race (questionnaire)		
	Upper GI	**Lower GI**	**Other**	**Upper GI**	**Lower GI**	**Other**
Runner 1			Dizziness (5;5) Urge to urinate (3)	Belching (2) Bloating (2) Nausea (2)	Flatulence (2)	Dizziness (5) Headache (2) Urge to urinate (4)
Runner 2		Flatulence (4;4;8)		Reflux (6) Bloating (6)	Flatulence (4)	
Runner 3	Nausea (3)			Nausea (3)	Urge to defecate (5)	Urge to urinate (4)
Runner 4		Side ache (6)		Reflux (9) Bloating (2)	Intestinal cramp (7) Flatulence (7) Urge to defecate (3) Side ache (8) Abdominal pain (2)	Urge to urinate (5)
Runner 5		Urge to defecate (5;5;4;3;3)		Reflux (4) Heartburn (4) Belching (5)	Intestinal cramp (4) Urge to defecate (5) Diarrhea (5)	

Upper GI, Upper respiratory and stomach complaints like: reflux, heartburn, belching, bloating, stomach cramps, vomiting, and nausea. Lower GI, Lower bowel complaints like: intestinal cramp, flatulence, urge to defecate, side ache, abdominal pain, loose of stool, diarrhea, and intestinal bleeding. Other, Dizziness, headache, muscle cramps, urge to urinate. During the race runners could score each 15 km potential complaints, in case athletes reported a complaint multiple times this is indicated by multiple scores between brackets (X;X). Each complaint that was mentioned received a score >0. If a complaint had a score >4, it was considered as serious.

maintain their total level of dehydration within a 2–3% range instead of the current 3.6%. Probably as a result of logistic challenges or impossibilities, most research groups reported only acute changes in body weight during exercise to estimate sweat rates and perturbations in hydration status without correcting for urine losses (20). This study adds to our knowledge that neglecting correcting for urine losses will overestimate sweat rate modestly (∼5–10%).

Carbohydrate intake is associated with greater endurance performance and capacity when compared with water alone (28). Additionally, higher carbohydrate intakes are associated with better performance. Therefore, ultra-endurance athletes are advised to consume up to 60–90 g of carbohydrates per hour (29, 30). Carbohydrate intake of 90 g/h or higher was reported earlier in individuals (17, 19), but in general, athletes consume lower amounts, which still allows them to finish the race (10–13, 16, 17). During the present study, two of the runners met the recommendation of 60 g/h (intakes of 62.6 and 62.5 g/h). The other three runners reported intakes between 27.1 and 43.7 g/h. Stellingwerff (19) estimated that an ∼60 kg world-class elite runner was oxidizing ∼3,250 kcal over a 100 km race; his estimation was based on glucose and glycogen representing ∼59% of the total metabolized energy (19). They also estimated that glycogen reserves would represent a maximal energy value of ∼2,000 kcal for a person of this weight. In the current study, we did not assess pre-race glycogen reserves or the pre-race diet, but only two out of five runners reported to adjust carbohydrate intake in the days before the race. Habitual carbohydrate intake in this type of runners is not necessarily high (17). Although speculative, we hypothesize that the glycogen storage potential should at least be equal in the current group of runners. Extrapolating the estimations of Stellingwerff (19) to the current race situations over 120 km, we speculate that runners should have an exogenous carbohydrate need of at least 375 g (∼30–38 g/h), which 4 out of 5 runners exceeded. The only runner observed with a lower carbohydrate intake was actually focusing on running the race with as few carbohydrates as possible with the idea of optimizing fat oxidation as much as possible. Although this person during the first 60 km consumed a very low amount of carbohydrates, he substantially increased his carbohydrate intake in the second part of the race. In the end, the runner consumed a total of 327 g of carbohydrates, which was lower than the estimated need during this type of race. However, it must be taken into account that the glycogen reserves might not be optimized before the race. Overall, runners did not meet the high 90 g/h recommendation. Most of them reported a constant hourly intake, and acknowledging that this type of exercise might actually have a lower carbohydrate need than frequently suggested by Stellingwerff (19), the consumed amount of carbohydrates over the course of the race was probably sufficient.

Several potential factors influenced food and fluid intake. First, runners were accompanied by a cyclist that could hand out all preferred products and drinks at the runner's request. This may not mimic typical food and fluid availability during other races, however, it provided an unique opportunity to get insight into food and fluid intake during an ultra-endurance event without being limited by the amount of aid stations. Secondly, it was relatively cold on the day of the race. We do not believe that the temperature influenced their original fluid intake strategy, as consumption was still within the normal range of previous reports (10–13, 16, 17). Third, the environmental conditions impacted the timing of food intake and fluid excretion because runners entered the four beach sections without their

cyclists. In all cases, runners were provided with one or more products before they entered the beach (total beach sections covered 24.5 km). Runners covered these sections within 40–60 min. This time without the cyclist therefore limited their regular feeding opportunity, as runners on average consumed food and/or fluid every 20 min. Finally, food and fluid intake might be affected by perceived GI complaints, as suggested by the literature (24). Fewer complaints were reported during the race, which suggests that there is no specific indication for a relation between running, food and fluid intake and GI complaints. We were able to compare the reported GI complaints during the race and the self-reported GI complaints afterwards using a questionnaire. The incidence and intensity of scored complains differed between both methods, in which the GI complaints during the race are perceived as less severe compared to the GI complaints reported after the race. Further research is necessary to investigate the relevance of this difference in reported GI complaints.

The strength of this study is the use of a continuous observational method to describe timing of carbohydrate and fluid intake during an ultra-endurance event. However, some limitations should be taken into account. First, one runner had a fecal stop and a second runner urinated one time on the beach. In both cases, we made a small correction to our calculation. Normally, gastrointestinal tract losses are small (∼100–200 mL/day) (20). In the case of the missing urine sample, we included the average urine excretion of this individual's other samples in the calculation. Second, we had a high drop-out rate (44%), which may have affected our overall results. A previous study, with a drop out race of 63%, described no difference between carbohydrate intake between finishers and non-finishers in a 161 km foot race (12).

In conclusion, runners were able to maintain a relatively constant hourly timing of food and fluid intake during the competition. The total level of dehydration exceeded the current recommendation (<2% dehydration over the course of the race). Products optimizing fluid retention in combination with a slightly higher absolute fluid intake should be considered for future races. Although none of the runners met the literature's suggested high carbohydrate recommendation of 90 g/h, these runners potentially have a lower carbohydrate need, but at least 2 out of 5 runners should consider raising their carbohydrate intake above 30 g/h during future competitions.

AUTHOR CONTRIBUTIONS

The study was designed by FW, DH, JV, NvdB, and KL. Data were collected by FW, DH, JV, NvdB, KL, and CB. The interpretation of data and preparation of the manuscript were done by FW, DH, and CB. All authors approved the final manuscript.

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Pharmacokinetics of β-Alanine Using Different Dosing Strategies

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Introduction: The ergogenic response following long-term ingestion of β-alanine shows a high inter-individual variation. It is hypothesized that this variation is partially caused by a variable pharmacokinetic response induced by inferior dosing strategies. At this point most supplements are either taken in a fixed amount (× g), as is the case with β-alanine, or relative to body weight (× g per kg BW), but there is currently neither consensus nor a scientific rationale on why these or other dosing strategies should be used. The aim of this study is to objectify and understand the variation in plasma pharmacokinetics of a single oral β-alanine dose supplemented as either a fixed or a weight-relative dose (WRD) in an anthropometric diverse sample.

Methods: An anthropometric diverse sample ingested a fixed dose (1,400 mg) ($n = 28$) and a WRD of β-alanine (10 mg/kg BW) ($n = 34$) on separate occasions. Blood samples were taken before and at nine time points (up to 4 h) after β-alanine ingestion in order to establish a pharmacokinetic profile. Incremental area under the curve (iAUC) was calculated by the trapezoidal rule. Plasma β-alanine was quantified using HPLC-fluorescence.

Results: The variation coefficient (CV%) of the iAUC was 35.0% following ingestion of 1,400 mg β-alanine. Body weight explained 30.1% of the variance and was negatively correlated to iAUC ($r = -0.549$; $p = 0.003$). Interestingly, the CV% did not decrease with WRD (33.2%) and body weight was positively correlated to iAUC in response to the WRD ($r = 0.488$; $p = 0.003$).

Conclusion: Both dosing strategies evoked an equally high inter-individual variability in pharmacokinetic plasma profile. Strikingly, while body weight explained a relevant part of the variation observed following a fixed dose, correction for body weight did not improve the homogeneity in β-alanine plasma response. We suggest to put more effort into the optimization of easy applicable and scientifically justified personalized dosing strategies.

Keywords: β-alanine, sports supplements, pharmacokinetics, personalized nutrition, carnosine

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INTRODUCTION

In sports nutrition, there is no uniformity in dosing strategies of ergogenic supplements to adults. A fixed dose (FD; × g per person per day) is by far the easiest to implement in daily practice and allows straightforward packaging and marketing. On the other hand, dosing is sometimes normalized for anthropometric characteristics. The weight-relative dose (WRD; × g per kg body weight per day), which is by far the most popular normalizing strategy, is somewhat less practical

to prescribe but may seem more adequate. The WRD is expected to correct for differences in body size and weight, which can easily differ by a factor 3 between, say, a female gymnast and a male American Football lineman. There is a striking paucity in the nutrition literature in general and in the sports nutrition literature in particular, relating to the pros and cons of these two, or any other normalization approach and even more so relating to the physiological differences and direct comparison of different approaches.

β-alanine (BA) is classified as a Group A supplement in the Sport Supplement Program of the Australian Institute of Sport[1] as it has been shown to improve performance of exercises lasting between 0.5 and 10 min (1). Its ergogenic effect is achieved by the intra-muscular increase of the dipeptide carnosine that is constituted from histidine and its rate-limiting precursor BA. Carnosine has multiple biochemical properties whereof its ability to buffer protons most likely is the major determinant that explains its ergogenic potential (2).

Currently, a FD is the most popular strategy in BA supplementation although different absolute doses are used (1). In the first report of BA as a human food supplement, Harris et al. investigated the pharmacokinetics of three different BA doses supplemented as WRD (10, 20, and 40 mg/kg BW) (3). Subsequently, these authors switched to FD and investigated the effect of a 15-d supplementation protocol of 3.2 and 6.4 g/d (FD) on muscle carnosine. In follow-up, Hill et al. (4) performed the first study on BA as an ergogenic food supplement with a FD (4–6.4 g/day). Thereafter (almost) all research, by this and other groups, was performed with absolute dosing strategies, but without further rationale.

As in many other supplementation studies, the improvement in exercise performance following BA ingestion is characterized by a lot of variation. For example, Saunders et al. (5) found that performance changes ranged from +0.0 to +72.7% (CV% > 60%) in distance covered in a YoYo intermittent recovery test level 2 (5). This is not surprising, as many others have shown that the variation in muscle carnosine loading is quite high as well. For example, Blancquaert et al. (6) found a change of gastrocnemius carnosine from 3.3% up to 119.0% (CV% = 74%) in people receiving 6.4 g BA for 23 days (6). As Hill et al. (4) showed that the change in muscle carnosine explains part of the change in performance, we can hypothesize that by reducing the variation in muscle carnosine increments, the ergogenic effect might also be more uniform. Interestingly, the coefficient of variation in BA's pharmacokinetic response was reported to be 32% in a standardized setting (7). As it was shown that muscle carnosine loading was correlated to plasma BA concentration in mice (8) and to fasted plasma BA concentrations after a supplementation period in humans [unpublished, data of Blancquaert et al. (6)], it is very likely that the variation in carnosine loading and therefore ergogenic response is explained, in part, by the variation in the acute pharmacokinetic profile of BA.

[1]Australian Sports Commission Group A sports supplement AIS. Available online at: https://www.ausport.gov.au/ais/nutrition/supplements/groupa (Accessed March 20, 2018).

As it is the objective of any sport supplement to optimize performance, it is obvious that athletes require correct and perhaps personalized dosing that induces a homogenous pharmacokinetic and ergogenic response. Providing a subtherapeutic dose might fail to affect performance, whereas supratherapeutic dosing might decrease performance or could even cause (unhealthy) side effects. In case of BA, low ingestion leads to the lack or low increases of carnosine, most likely insufficient to affect performance (4, 9). On the other hand, a supratherapeutic dose of BA can acutely cause discomfort in the form of paraesthesia (3). Due to the acute side-effects of BA, a slow release formula was developed in order to reduce peak concentration but maintain total pharmacokinetic response (AUC) (7). Some have also suggested that chronic ingestion of high doses of BA can cause a decline in muscle and plasma histidine (6, 10), whereby a negative effect on health or performance cannot be excluded. On the other hand, others did not observe a decline in muscle histidine following chronic BA supplementation (3, 11). In general, one could expect that with a FD the smaller/lighter people will be more prone to overdosing and the larger/heavier people to underdosing. However, it might equally be that with WRD the reverse is occurring (smaller people get underdosed and larger people overdosed). The latter was suggested for carbohydrate ingestion and bioavailability during exercise since it was argued that this is more dependent on GI tract characteristics rather than on body weight thereby pleading in favor of a FD for carbohydrates (12).

Summarized, there is at this point no scientific rationale to use a FD in BA supplementation. On the other hand, there is no information why other dosing strategies should be used. Based on the current knowledge there is no way to know which dosing strategy evokes the most homogenous plasma response. Therefore, the aim of this study is to objectify and to understand the variation in plasma pharmacokinetics of a single oral BA dose (FD or WRD) in an anthropometric diverse sample.

METHODS

Subjects

Thirty-four subjects (age 25.1 ± 4.29 y; body weight: 70.4 ± 14.9 kg; height: 1.73 ± 0.11 m); volunteered to participate in the first part of this study (WRD) whereof 28 (age: 24.8 ± 4.25 y; body weight: 68.1 ± 15.2 kg; height: 1.72 ± 0.11 m) completed the second part (FD) (**Table 1**). All subjects were in good health and none of the participants was vegetarian. Both males and females (WRD: 19 males/15 females and FD: 14 males/females) from a mixed ethnic background were recruited. The study protocol was approved by the local ethical committee (Ghent University Hospital, Belgium) and written informed consent was obtained from all participants before the study.

Study Design and Sample Collection

Subjects arrived fasted at the lab at two separate occasions. At arrival, a catheter was inserted in an antecubital vein and the first blood sample was taken (heparin tube). Hereafter, a standardized breakfast, consisting of white bread, hazelnut paste (Nutella) and semi skimmed milk, was consumed. The total

TABLE 1 | Anthropometric characteristics sample.

		FD (1,400 mg)	WRD (10 mg kg^{-1})
N	Men	14	19
	Women	14	15
Age (year)	Min	18	18
	Max	34	35
	Mean	24.8	25.1
	SD	4.3	4.3
Body weight (kg)	Min	46.0	46.0
	Max	104.5	104.5
	Mean	68.1	70.4
	SD	15.2	14.9
Height (m)	Min	1.50	1.50
	Max	1.94	1.94
	Mean	1.72	1.73
	SD	0.11	0.11

FD, fixed dose; WRD, weight-relative dose.

calorie count of the breakfast was 23% (388 ± 50 kcal) of the resting metabolic rate (RMR) as calculated by the formula of Mifflin (13) (1,672 ± 204 kcal). Ten minutes after the start of the breakfast, a single dose of pure BA (Indis nv, Belgium) in gelules was ingested with water. Consecutively nine blood samples were taken after 20, 40, 60, 90, 120, 150, 180, 210, and 240 min. During the experiment, subjects drank water *ad libitum* and they refrained from any physical activity. All participants received a relative dose of 10 mg/kg body weight pure BA on the first test day, whereas 28 of the 34 participants returned for a second experimental test day where they received an absolute dose of 1,400 mg. To obtain information about the subjective feelings and the location of paraesthesia, the subjects received a standardized questionnaire at the end of every experimental day asking for the occurrence, intensity, localization, timing and description of possible discomfort/side-effects (7).

The absolute dose of 1,400 mg corresponded to a relative dose of 20.79 ± 4.45 mg/kg BW (range: 13.40–30.43 mg/kg BW), whereas the relative dose matched 703.5 ± 149.4 mg (range: 460–1,045 mg). Standardized allocation of experimental test days was used in order to test for potential paraesthesia occurring with the lower dose (WRD), before exposing subjects to a higher dose (FD).

Determination of Plasma Metabolites by High-Performance Liquid Chromatography

Heparin plasma samples were analyzed for BA using a previously published HPLC method (6). In short, heparin plasma samples were deproteinized using a 1:9 ratio of 35% sulfosalicylic acid. Plasma supernatant was mixed with ACCQ Fluor Borate buffer and Fluor reagent from the AccQtag Chemistry kit (Waters sa-nv, Belgium) in a 1:7:2 ratio. Standard solutions of BA were treated similarly before HPLC analyses. The derivatized samples were applied to a Waters Alliance HPLC system with the following parameters: XBridge BEH column (4.6 × 150 mm, 2.5 μm; Waters) heated to 37°C; fluorescence detector (excitation/emission wavelength: 250/395 nm); using a flow gradient containing different amounts of buffer A (10% eluent A [Waters], 90% ddH2O), buffer B (100% acetonitrile), and buffer C (100% ddH2O) at a flow rate of 1 mL.min^{-1}.

Pharmacokinetics and Statistical Analysis

Pharmacokinetics was investigated using a first order kinetic and non-compartmental model. Incremental area under the curve (iAUC) was calculated by subtracting the baseline of the AUC calculated using the trapezoidal rule. Cmax was determined as the maximal concentration measured, whereas Tm was determined as the time Cmax was reached. $T_{1/2}$ was calculated as 0.693 divided by the elimination constant (k_e), whereas the k_e was computed as −2.303 multiplied by the slope of the individual linear curve of the log10 from Cmax till the concentration at time 240 min (14). CV% (standard deviation divided by mean) was calculated for all variables. Before statistical analysis, normality of the continuous variables was checked using Shapiro-Wilk. Multivariate analyses of variance (MANOVA) was performed to determine the role of sex on the different pharmacokinetic parameters following FD. Pearson correlations and linear regression were performed between the anthropometric and pharmacokinetic parameters. All statistical analyses were performed using the Statistical Package for the Social Sciences (version 25.0; SPSS, Chicago, IL). Values are presented as mean ± SD and significance was assumed at $P \leq 0.05$.

RESULTS

Variability With FD

The inter-individual variability in iAUC and Cmax that could be observed in BA plasma pharmacokinetics following ingestion of a single FD of 1,400 mg BA was 35.0% (18,550 ± 6,495 μM.min; range: 9,334–38,183) and 40.2% (218.4 ± 87.9 μM; range: 91.7–440.5), respectively (**Figure 1A**) (**Table 2**). The $T_{1/2}$ varied from 32.6 to 97.8 min and Tm varied from 40 to 150 min. These pharmacokinetic parameters were not significantly different between sexes (data not shown).

Anthropometric Factors Underlying the Variation With FD

The iAUC of the FD was negatively correlated with body weight, height and RMR, but not to BMI (**Figure 2**). The strongest correlation with plasma iAUC was found for height, explaining 33.8% of the variance, which was only slightly higher than the 30.1% explained by body weight (**Table 3**). Cmax of FD was also correlated to all anthropometrics (**Table 3**). Body weight had the highest explained variance (29.1%) for Cmax.

Variability With WRD

We also investigated the pharmacokinetic response following ingestion of 10 mg/kg BW BA (WRD). Despite this body weight correction of dosing, a nearly equally high inter-individual variability was observed (**Table 2**; **Figure 1B**) and the fold-difference between the highest and lowest responder was even

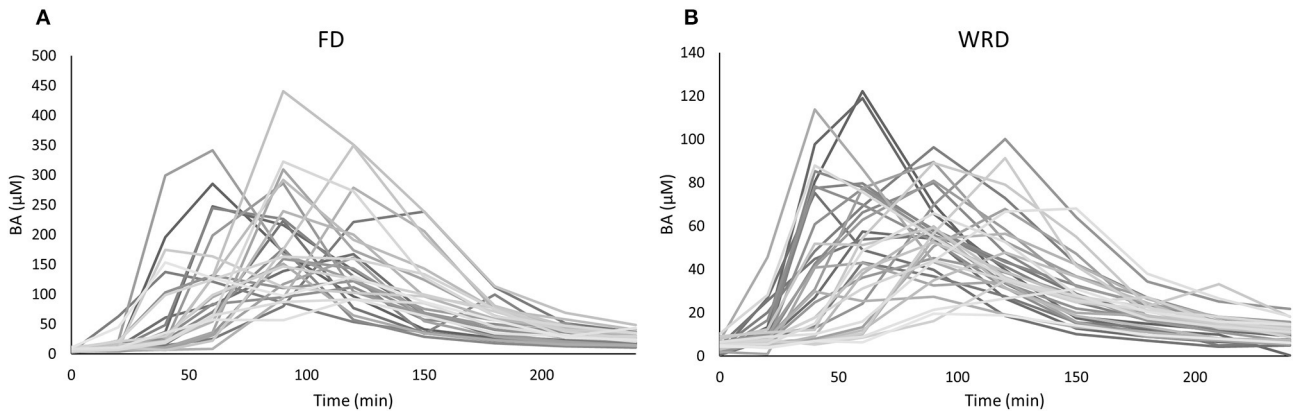

FIGURE 1 | Variability in plasma pharmacokinetics in an anthropometric diverse sample following a **(A)** fixed (FD) and **(B)** weight-relative dose (WRD). Plasma β-alanine (BA) concentrations (μM) were determined before and at nine time points after the ingestion of 1,400 mg **(A)** or 10 mg/kg BW **(B)** BA. The observed variation in iAUC is 35.0 and 33.2%, respectively.

TABLE 2 | Observed variation in iAUC and Cmax in both dosing strategies.

		FD	WRD
iAUC	%CV	35.0	33.2
	Fold difference (highest vs. lowest)	4.09	7.16
Cmax	%CV	40.2	37.5
	Fold difference (highest vs. lowest)	4.81	6.25

FD, fixed dose; WRD, weight-relative dose.

more pronounced. The CV% of iAUC and Cmax was 33.2% (6,134 ± 2,038 μM.min; range: 1,424–10,201) and 37.5% (69.0 ± 25.9 μM; 19.6–122.2 μM), respectively.

The iAUC of the WRD was positively correlated to body weight, height, BMI, and RMR (**Figure 3**), while Cmax was correlated to body weight and RMR but not to height and BMI (**Table 3**).

Relationship Dose and iAUC

As the FD and WRD were in a different absolute dose range, we investigated the link between dose and iAUC. There was a non-linear relationship between the dose and the iAUC on both the individual and the population level (**Figure 4**).

Side Effects

Nobody reported paraesthesia following WRD and two subjects reported paraesthesia following ingestion of FD. These subjects did not have the highest values of AUC, Cmax, Tm, or $T_{1/2}$ of the evaluated population, but the moment of paraesthesia matched their individual Cmax value.

DISCUSSION

In this study, the inter-individual differences in pharmacokinetic response (iAUC) following BA ingestion are described. The iAUC is a crucial parameter because it reflects the concentration and duration of elevated plasma BA. It is assumed that the variation in acute BA pharmacokinetic profile reflects the variable response on carnosine loading and thereby ergogenic outcome following long-term BA supplementation. The coefficient of variation of the iAUC was 35.0% following a FD (**Figure 1A**), with a marked 4-fold difference between the lowest and highest iAUC. This variation resembles the 32% variation reported by Décombaz et al. (7). Seeking for an explanation for the high diversity in physiological response, it was observed that body weight explained a relevant part (30.1%) of the variability in plasma kinetic response. The negative correlation to body weight ($r = -0.549$; $p = 0.003$) signifies that heavy people received too little and light people received too much BA, to cause a homogenous response. The current observations call for a body weight correction to personalize dosing.

The striking observation of the current study is that the body weight correction (10 mg/kg BW) did not improve homogeneity in BA plasma response, with a CV% of 33.2% and an even more pronounced 7-fold difference between lowest and highest iAUC (**Figure 1B**). Instead of a negative correlation, there was now a positive correlation with body weight (r =0.488; $p = 0.003$). This means that when trying to correct the dose for body weight, the problem is reversed, thereby overdosing the heavy people and underdosing the less heavy people. Thus, although the principle of weight-corrected dosing seemed valid for BA, we simply replaced one problem by an equally large new problem, leading to zero progression toward homogeneity and individualized supplementation.

One possible explanation for the failure to improve homogeneity of supplement response could be that body weight is not the optimal scaling factor for dose calculation. Other body dimensions might more accurately reflect determinants of the pharmacokinetic response through for example GI tract dimension and therefore absorption surface, liver volume and thereby BA degradation capacity (8), kidney volume (15), blood volume (16), etc. However, other parameters, such as height, BMI, or RMR (**Figure 2**; **Table 2**) did not yield a better explained

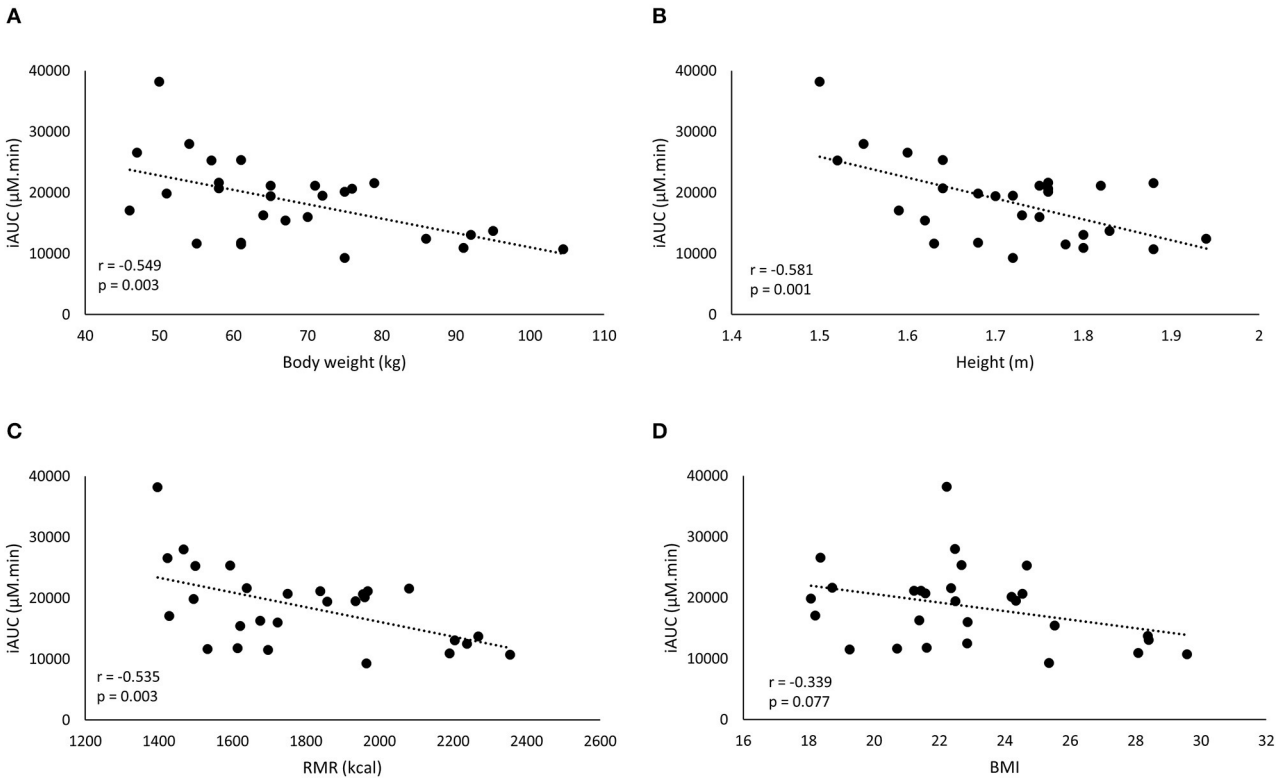

FIGURE 2 | A negative correlation is observed between iAUC of the fixed dose and the anthropometric characteristics: body weight (BW) **(A)**, height **(B)**, resting metabolic rate (RMR) **(C)**, and BMI **(D)**.

TABLE 3 | Correlations between anthropometrics vs. iAUC and Cmax.

		FD		WRD	
		iAUC	**Cmax**	**iAUC**	**Cmax**
BW	*r*	−0.549*	−0.540*	0.488*	0.360*
	r^2	0.301	0.291	0.238	0.130
Height	*r*	−0.581*	−0.531*	0.415*	0.313$^{\$}$
	r^2	0.338	0.282	0.172	0.098
BMI	*r*	−0.339$^{\$}$	−0.378*	0.401*	0.289$^{\$}$
	r^2	0.115	0.143	0.161	0.083
RMR	*r*	−0.535*	−0.522*	0.479*	0.364*
	r^2	0.287	0.273	0.230	0.132

FD, fixed dose; WRD, weight-relative dose $^*p < 0.05$; $^{\$}0.05 < p < 0.10$.

variance than body weight in the current study. Therefore, we believe that, when scaling is appropriate, body weight might still partially be a relevant scaling factor, as it is the easiest to translate and adopt to the population.

Since both the pharmacokinetic response of the FD and WRD suffered equally from an influence of body weight, yet in opposite directions, we now propose that supplement dosing should only partially be scaled to body weight. One approach would be to consider 50% of the dose to be given as a FD and 50% as a WRD, with a 70 kg person as a reference. Transferred to the example of a targeted 1,400 mg or 20 mg/kg BW BA, a 70 kg person would receive 700 mg as FD and 700 mg as 10 mg/kg body weight. Thus, with this scheme (700 mg as FD and 10 mg/kg BW as WRD), this would result in a 1,200 mg dose for a 50 kg person and 1,700 mg for a 100 kg person. An alternative approach would be to normalize the entire dose to only a portion of the body weight. When comparing anthropometrically diverse populations, scaling maximal oxygen uptake to body weight is usually done by dividing VO2 by 2/3rd of the lean body mass (17). The latter is less likely to be easily adoptable because it requires lean body mass determination and calculation beyond the general public's abilities. It is important to consider that, the dose-scaling strategies presented here can only be used as a suggestion since these were not tested in this study.

Another interesting observation was that the iAUC was non-lineary related to the dose (**Figure 4**). This implies that especially with higher dosage, a certain change of dose will not induce the same effect on the iAUC. In the former example this means that when a 100 kg person ingests a WRD of 20 mg/kg BW (2,000 mg) or a partially FD and WRD of 700 + 10 mg/kg BW (1,700 mg) this would result in a 87 and 40% increase of iAUC while the dose only increased 43 and 21%, respectively, compared to a FD of 1,400 mg. The fact that the dose is non-linearly related to iAUC adds an extra layer of complexity in future research investigating different dosing strategies.

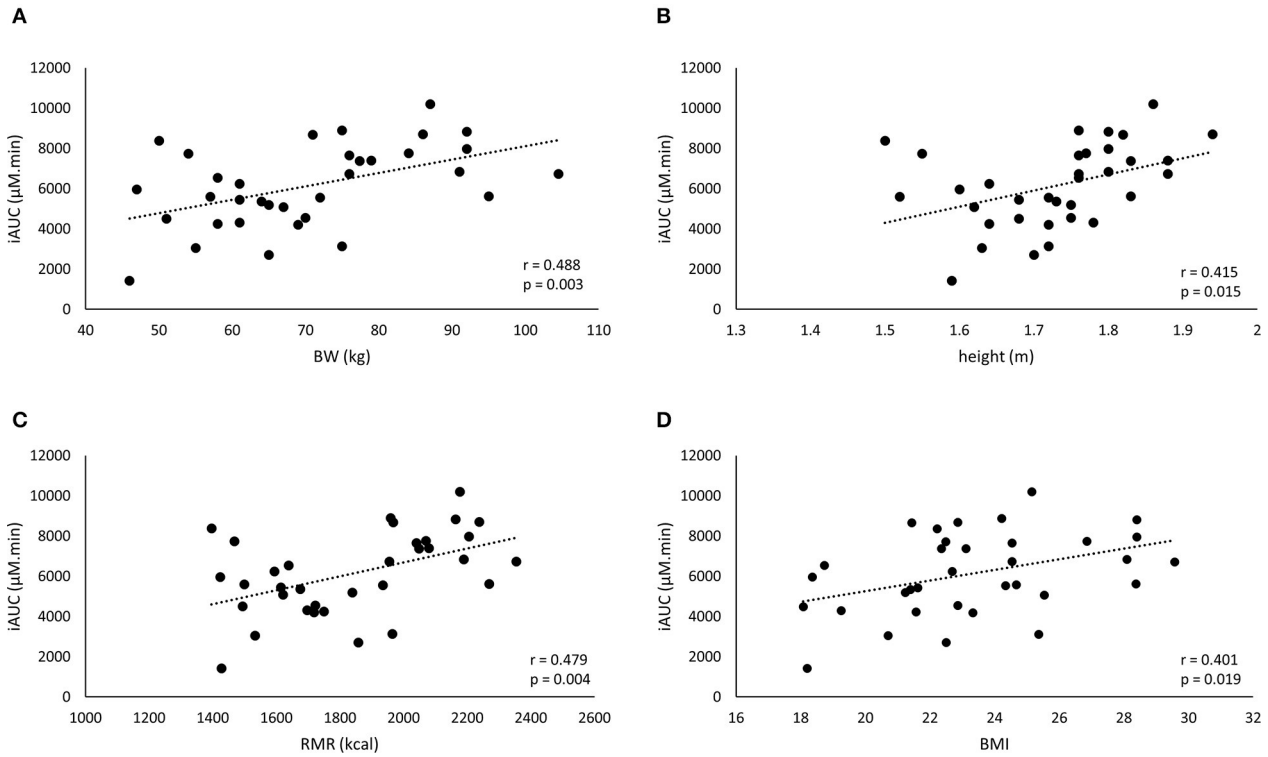

FIGURE 3 | A positive correlation is found between the iAUC of the weight-relative dose and the anthropometric characteristics: body weight (BW) **(A)**, height **(B)**, resting metabolic rate (RMR) **(C)**, and BMI **(D)**.

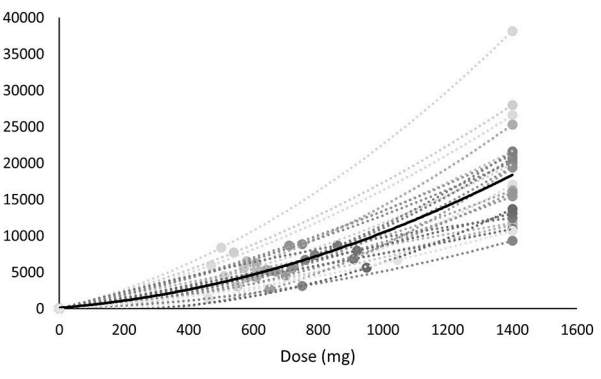

FIGURE 4 | The iAUC of β-alanine is non-linearly related with the supplemented dose. The mean is shown in black, whereas the individual values are shown in gray.

In the current experiment, a quite diverse sample was included consisting of men and women, from different ethnicities and with different anthropometric characteristics. The current sample somewhat resembles the population toward which results should be generalized, although our sample did not include people above 105 kg and 1m94, which is a limitation. Current standard practice is to supplement athletes with a fixed dose. In this sample, the fixed dose of 1,400 mg resembled a relative dose of 13 and 30 mg/kg BW for the heaviest and lightest subjects respectively. A WRD of 30 mg/kg BW is already quite high when considering the pioneering study reported significant side-effects, although not recorded as unpleasant, at a dose of 20 mg/kg BW (3). On the other hand, including more heavy subjects would most likely have resulted in an even smaller physiological response when ingesting the fixed dose (<13 mg/kg BW). In future research, it is the scientific community's responsibility to test and formulate recommendations for all athletes, also those with more extreme anthropometric characteristics.

Although other supplements are characterized by a different metabolism, similar dose related considerations have not yet been made for the other evidence-based performance supplements. Within each supplement there exists a more or less standard practice, with bicarbonate and caffeine being administered in WRD and creatine and nitrate mostly used in FD. Interestingly, it seems that the adopted dosing strategy is primarily based on the pioneering study. Jones et al. (18) were the first to investigate bicarbonate as an ergogenic supplement and used a WRD (0.3 g/kg), whereafter the community continued to do so. Creatine and nitrate are mostly used in FD in accordance with the pioneers who supplemented a FD of 5 g/dose, 4–6 times a day (3), and 500 ml/day beetroot juice (NO^{3-}:5.5 mmol/day) (19) to improve exercise, respectively. In contrast, pioneering researchers used caffeine in a FD of 330 mg (20), but the scientific community shifted toward the use of WRD. One might suggest that, at this point, WRD is mostly used in ergogenic aids that are taken acutely and whereby overdosing might cause ergolytic

effects. In contrast, convenient FD is used when supplements are chronically administered and when acute side effects are not likely to affect performance. In any case, there is at this point no scientific justification on why current dosing strategies are used in relation to optimization of personalized responses.

In the present study, a FD and WRD of 1,400 mg and 10 mg/kg BW were used, respectively. The FD of 1,400 mg corresponded to a relative dose of 13.40–30.43 mg/kg BW, whereas the WRD matched 460 mg up to 1,045 mg. These doses and the corresponding physiological response (iAUC) are different, making direct comparison between two dosing strategies used in this investigation impossible. We acknowledge this difference in absolute dose between de FD and WRD conditions as a limitation to the current study. Nonetheless, we deem the conclusions of the current data as valid and valuable.

In summary, the current study showed that—at least for the example of BA—neither FD nor WRD is adequate toward personalized nutrition, since in both cases the observed variation was equally high and partially explained and correlated to anthropometrics. This underscores the importance to better address the relationship between different doses of nutritional supplements and the physiological responses they elicit in an anthropometric diverse sample. Future studies will need to test more advanced dose-scaling strategies, where after it will be possible to provide scientifically based recommendations for provoking more homogenous physiological responses in athletes.

AUTHOR CONTRIBUTIONS

JS, IE, and WD conceived and designed the experiment. JS, FL, and IE performed the experiment. JS, IE, and WD analyzed and interpreted the experiment. JS, IE, FL, and WD wrote and approved the final version of the manuscript.

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Effects of Caffeine Ingestion on Anaerobic Capacity in a Single Supramaximal Cycling Test

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The aim of this study was to verify the effects of caffeine on anaerobic capacity estimated by the sum of the estimated glycolytic [$E_{[La]}$] and phosphagen [E_{PCr}] metabolism based on blood lactate and excess post-oxygen consumption responses ($AC_{[La-]+EPOCfast}$). Fourteen male cyclists were submitted to a graded exercise test to determine the maximal oxygen uptake ($\dot{V}O_{2max}$) and intensity associated with $\dot{V}O_{2max}$ ($i\dot{V}O_{2max}$). Subsequently, the participants performed two supramaximal efforts at 115% of $i\dot{V}O_{2max}$ to determine the $AC_{[La-]+EPOCfast}$, after previous supplementation with caffeine (6 mg·kg^{-1}) or a placebo (dextrose), in a cross over, randomized, double blind, and placebo-controlled design. The time to exhaustion was higher in the caffeine (186.6 ± 29.8 s) than in the placebo condition (173.3 ± 25.3 s) ($p = 0.006$) and a significant correlation was found between them ($r = 0.86$; $P = 0.00008$). Significant differences were not found between $AC_{[La-]+EPOCfast}$ values from the placebo (4.06 ± 0.83 L and 55.2 ± 5.7 mL·kg^{-1}) and caffeine condition (4.00 ± 0.76 L and 54.6 ± 5.4 mL·kg^{-1}); however, a significant correlation was observed only for $AC_{[La-]+EPOCfast}$ expressed in absolute values ($r = 0.74$; $p < 0.002$). The $E_{[La]}$ and E_{PCr} also presented no significant differences and they were significantly correlated ($r = 0.82$ and $r = 0.55$, respectively; $p < 0.05$). We conclude based on the overall comparison of mean values between two treatments that acute caffeine ingestion improves the time to exhaustion but does not affect anaerobic capacity estimation.

Keywords: caffeine, anaerobic capacity, performance, running, ergogenic aids

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INTRODUCTION

Acute caffeine ingestion has been shown to cause an increase in the excessive post-exercise oxygen consumption (EPOC) after resistance training (1), time to exhaustion during supramaximal effort (2) and other performance parameters. Moreover, the improvement in time to exhaustion during a supramaximal effort after caffeine intake caused by changes in oxygen consumption ($\dot{V}O_2$)at the exhaustion moment, possibly affecting the phosphagen energy system estimation (2). In addition, some studies have shown that acute ingestion of caffeine increased time to exhaustion during a supramaximal test and anaerobic capacity estimated by the maximal accumulated oxygen deficit (MAOD) (3–5). Bell et al. (3) found a consequent increase in peak lactate concentration after a

supramaximal effort to determine MAOD with caffeine supplementation. However, other studies have not shown significant effects of caffeine ingestion on exercise blood lactate concentration after supramaximal efforts to determine MAOD (4, 5). The possible changes in lactate concentrations could cause changes in the estimation of glycolytic system and consequently alter the estimation of capacity anaerobic when estimated by blood lactate concentration and EPOC responses (6–8), and consequently can alters the anaerobic capacity.

MAOD is one of the most accepted methods for evaluating an individual's maximal capacity of ATP resynthesis by means of the non-mitochondrial metabolism, which is determined by means of calculations involving $\dot{V}O_2$ measurements during several submaximal exercise sessions and one supramaximal exhaustive exercise session (9). However, the time-consuming nature of MAOD determination can discourage its use in routine athlete training and, for this reason, the anaerobic capacity has been alternatively estimated using only a single supramaximal effort ($AC_{[La-]+EPOCfast}$) (6–8). $AC_{[La-]+EPOCfast}$ determination is based on an estimation of the oxygen equivalent from the glycolytic ($E_{[La-]}$) and phosphagen (E_{PCr}) pathways, considering the accumulated blood lactate (10) and the fast component of the excessive post-exercise oxygen consumption ($EPOC_{fast}$) (11), respectively. The $AC_{[La-]+EPOCfast}$ is an advantageous method for anaerobic capacity assessment and its validity has been demonstrated by the absence of significant differences with the conventional MAOD determined in cycling (6, 7), running (8), and table tennis (12, 13). Furthermore, $AC_{[La-]+EPOCfast}$ method has shown high reliability (intraclass correlation coefficient [ICC] = 0.87 and typical error = 0.27 L) and significant associations with mechanical variables assessed in a 30 s maximal effort (14), besides to be sensitive in distinguishing individuals with different physical conditioning status (15).

The $E_{[La-]}$ is estimated by the values of blood lactate elevation from baseline during exercise, which could be influenced by factors related to the efflux of this metabolite from the muscle (16). On the other hand, the E_{PCr} is estimated by computing the $EPOC_{fast}$, which could be affected by any factor that causes alterations in the parameters related to this metabolic pathway calculation (e.g., oxygen uptake attained at exhaustion) (17). Thus, any intervention capable of modifying these responses (i.e., blood lactate and post-exercise oxygen consumption) could compromise the reliability of the $AC_{[La-]+EPOCfast}$. For example, the $AC_{[La-]+EPOCfast}$ was increased after acute supplementation with sodium bicarbonate due to $E_{[La]}$ alteration (16). However, it is not yet clear whether $AC_{[La-]+EPOCfast}$ is sensitive to possible changes caused in the $EPOC_{fast}$, induced for example by caffeine ingestion (1, 2).

De Poli et al. (2) found no effect of caffeine on $AC_{[La-]+EPOCfast}$ values determined in running, but suggested possible alterations in the relative contributions of the glycolytic (+9.3% with ∼68% possibly positive effect) and phosphagen metabolic (−5.4% with ∼76% possibly negative effect) pathways. Nevertheless, anaerobic evaluations are widely used in cycling (18) and investigations into the effects of caffeine on cycling $AC_{[La-]+EPOCfast}$ are missing. Importantly, different physiological responses can be observed in cycling compared to running (19) and MAOD seems to be affected by the exercise mode (20). Thus, considering the possible "fluctuations" in determining $AC_{[La-]+EPOCfast}$ that could be caused by these changes in E_{PCr} and $E_{[La]}$, it is necessary to investigate the effects of acute caffeine intake on the estimation of "anaerobic" capacity using this protocol in cycling.

Therefore, the aim of the present study was to verify the effects of acute caffeine ingestion on "anaerobic" capacity estimated by the $AC_{[La-]+EPOCfast}$ in cycling. We hypothesized that caffeine intake would improve performance in the supramaximal effort and change the relative energetic contribution of $E_{[La-]}$ and E_{PCr} on $AC_{[La-]+EPOCfast}$ method, as observed in running (2).

MATERIALS AND METHODS

Subjects

Fifteen male mountain bikers were considered eligible to participate in the study. Participants were recruited from regional cycling groups. To be included, they should be healthy, without any vascular disease, metabolic disorders, recent muscle-skeletal, or joint injuries and should not have used nutritional supplements as beta-alanine and creatine or pharmacological substances for at least 3 months. One participant was excluded from the study due to the inclusion criteria and therefore the final sample size was composed of 14 bikers. Five subjects had been competing at the regional level for at least 10 years. The other nine subjects reported at least 1 year of regular training and competition experience. The average weekly training volume reported by these individuals was 203 ± 122 km per week with a training frequency of 3–6 times.

One individual was excluded from the study due to high self-reported habitual daily caffeine ingestion (∼780 mg·day^{-1}). The participants' daily intake of caffeine was estimated for 3 days prior to the commencement of the study and reported to be 53.4 ± 39.8 mg·day^{-1}. All subjects were prohibited to consume any food or drink containing caffeine (i.e., tea, coffee, soft drinks, energy drinks, chocolate, and others) and alcohol, as well as were instructed to refrain to perform vigorous physical activity for at least 24 h before each test session. In addition, all subjects were instructed to consume their habitual meal. The characteristics of the subjects are presented in **Table 1**.

The subjects were informed about the risks and benefits of the procedures and signed a written consent prior to commencing study participation. All procedures were approved by the local Ethics Committee (Protocol 645 784/2014) and were conducted in accordance with the Helsinki Declaration.

Abbreviations: $[La^-]$, blood lactate concentration; $[La^-]_{Peak}$, peak of blood lactate concentration; $[La^-]_{Rest}$, rest blood lactate concentration; $AC_{[La-]+EPOCfast}$, anaerobic capacity alternative estimation; $E_{[La-]}$, glycolytic pathway; E_{OXID}, oxidative metabolism contribution; E_{PCr}, phosphagen pathway; $EPOC_{fast}$, fast component of the excessive post-exercise oxygen consumption; GXT, graded exercise test; HR, heart rate; $i\dot{V}O_{2max}$, intensity associated with maximal oxygen uptake; MAOD, Maximal accumulated oxygen deficit; RER, Respiratory exchange ratio; RPE, ating of perceived exertion; VO_2, oxygen consumption; $\dot{V}O_{2max}$, maximal oxygen uptake; SWC, Smallest worthwhile change

Experimental Design

The study design was a placebo-controlled double-blind crossover randomized trial. **Figure 1** presents a flow diagram of the study. Initially, the subjects were submitted to a graded exercise test (GXT) to determine the maximal oxygen uptake ($\dot{V}O_{2max}$) and intensity associated with $\dot{V}O_{2max}$ ($i\dot{V}O_{2max}$). Next, they performed two supramaximal efforts at 115% of $i\dot{V}O_{2max}$ to determine the $AC_{[La-]+EPOCfast}$, with or without (placebo condition) caffeine supplementation. The three sessions were separated by a minimum of 48 h. In all tests, the warm-up was standardized at 100 W for 5-min and was carried out 5-min before the tests.

All exercise tests were performed on an electromagnetic cycle ergometer (Lode-Excalibur, Lode, Netherlands). The subjects were instructed to adopt a preferred cadence between 70 and 90 rpm and to maintain the chosen cadence with a maximum variation of ± 5 rpm throughout the tests. The procedures for each study session were applied in an environment with

TABLE 1 | Characteristics of the subjects (n = 14).

	Age (years)	Height (cm)	Body mass (kg)	Lean mass* (kg)	LM-LL* (kg)	Fat*(%)
Mean ± SD	30 ± 6	179.4 ± 8.4	73.2 ± 11.4	58.3 ± 8.5	20.9 ± 3.4	15.4 ± 4.9

*Values are mean ± SD. LM-LL, Lean mass of lower limbs. * measured by DXA (Discovery, Hologic, USA).*

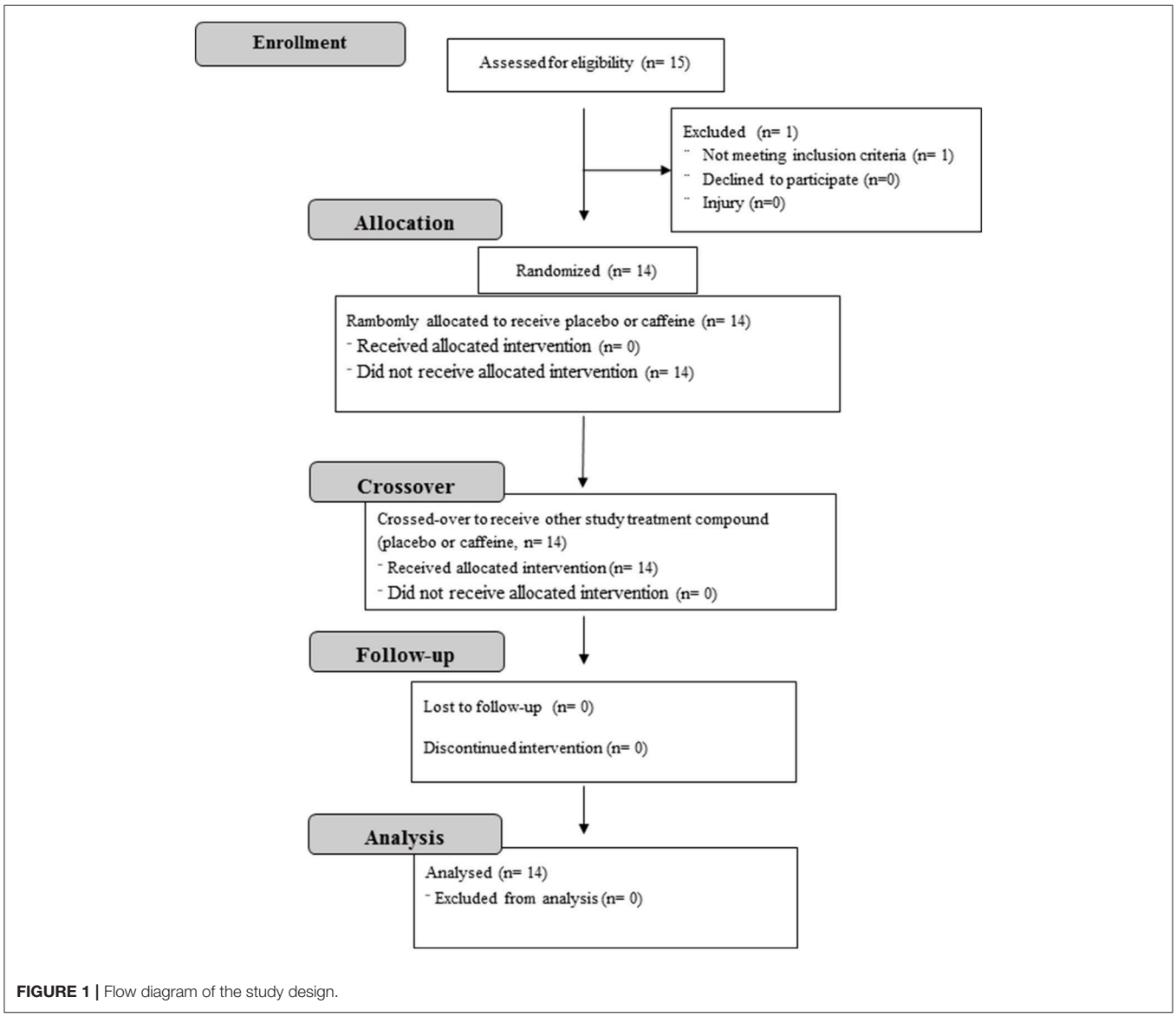

FIGURE 1 | Flow diagram of the study design.

controlled temperature and humidity (20 ± 1°C and 61 ± 8%, respectively).

For all laboratory visits, the subjects were informed to maintain the normal diet during the day and to make a meal between 2 and 4 h before to start the exercise procedures.

Physiological and Metabolic Data Collection

The respiratory responses were measured breath-by-breath by a stationary gas analyzer (Quark CPET, COSMED, Rome, Italy). The gas analyzer was calibrated before each test session using gas samples with known concentrations (5.00% CO_2 and 16.02% O_2, White Martins®, Osasco, Brazil) and room air, while the turbine was calibrated through a 3-L syringe (Hans-Rudolf, USA). The $\dot{V}O_2$ obtained during the tests was smoothed each 5 points and interpolated at 1 s intervals through OriginPro 9.0 software (OriginLab Corporation, Microcal, Massachusetts, USA). The heart rate (HR) was measured by a transmitter belt with wireless connection to the gas analyzer (Wireless HR 138 Monitor, COSMED, Rome, Italy),while the rating of perceived exertion (RPE) was assessed using the 6–20 Borg scale (21).

The blood lactate concentration ($[La^-]$) was measured from blood samples collected from the ear lobe (25 μl) at rest ($[La^-]_{Rest}$) (prior to warm-up) and 3, 5, and 7 min after each maximal test to determine the peak lactate concentration ($[La^-]_{Peak}$). The blood samples were collected and stored in *Eppendorf* tubes containing 50 μL of 1% sodium fluoride and then analyzed using a YSI 2300 STAT *(Yellow Spring Instruments, Ohio, USA)* (typical error of ± 2%).

The subjects remained seated for 10 min to determine the $\dot{V}O_2$ baseline and $[La^-]_{Rest}$. Baseline $\dot{V}O_2$ was considered as the mean of the final 2 min, while the exhaustion $\dot{V}O_2$ was considered the mean of the final 30 s of the supramaximal test.

Body Composition Analysis

Body composition was measured by dual-energy X-ray absorptiometry (DXA) using the Discovery corporal scanner (Hologic, Sunnyvale, USA). The body segmentation analysis was carried out with the horizontal line positioned above the bowl slightly above iliac crest. The angular lines that define the pelvic triangle were sectioned at the femur, and the vertical line positioned between the legs dividing the two feet. The lean mass of the lower limbs (LM-LL) was considered the sum of the right and left legs, not considering the bone mass values (7).

Graded Exercise Test (GXT)

The GXT was designed to induce exhaustion in ∼8–12 min (22). The initial power output was set at 100–150 W with increments of 25 W every 2 min until voluntary exhaustion or the inability to maintain the pre-defined cadence (23). In each test stage, $\dot{V}O_2$ measured during the final 30 s was averaged. The highest 30 s average $\dot{V}O_2$ obtained during the test was considered as the $\dot{V}O_{2max}$, considering the verification of a plateau in $\dot{V}O_2$ (variation in $\dot{V}O_2 < 2.1$ mL·kg^{-1}·min^{-1} between the final and penultimate stage of exercise). Secondary criteria were: maximal HR ≥ 90% of predicted maximal value, respiratory exchange ratio (RER) ≥ 1.10, and peak lactate ≥ 8.0 mmol·L^{-1} (22). The i$\dot{V}O_{2max}$ was assumed as the lowest intensity at which the $\dot{V}O_{2max}$ was attained (24).

Caffeine Supplementation and Supramaximal Efforts

The subjects ingested 6 mg·kg^{-1} of caffeine or a placebo (dextrose) (Neonutri, Minas Gerais, Brazil) 1 h before each supramaximal effort (Gemini Pharmaceutical Ingredients Industry Ltda, Anápolis, GO, Brazil), in a double blind and randomized fashion. The caffeine and placebo were contained in identical gel capsules, which were produced in our laboratory using a manual capsule filling machine. The caffeine dosage used was chosen as it has been proved to cause changes in the excessive post-exercise oxygen consumption (1).

The individuals performed the supramaximal efforts at 115% of i$\dot{V}O_{2max}$ to determine the time to exhaustion at this intensity and the corresponding $AC_{[La-]+EPOCfast.}$. The E_{PCr} was estimated by the $EPOC_{FAST,}$ analyzed using a bi-exponential model (Equation 1) (Origin PRO 9.0, OriginLab Corporation, Micrical, Massachusetts, USA) and corresponded to the product between A_1 and τ_1 (Equation 2) (6–8). The $E_{[La]}$ was estimated by the difference between the $[La^-]_{Peak}$ and $[La^-]_{Rest}$, considering each 1.0 mmol·L^{-1} of accumulated lactate equivalent to 3 mL·kg^{-1} of oxygen (10). The $AC_{[La-]+EPOCfast}$ was determined from the sum of oxygen equivalents of $E_{[La-]}$ and E_{PCr} pathways (Equation 3)

$$\dot{V}O_{2(t)} = \dot{V}O_{2(Rest)} + A_1\left[e^{-\left(\frac{t}{\tau 1}\right)}\right] + A_2\left[e^{-\left(\frac{t}{\tau 2}\right)}\right] \quad (1)$$

$$E_{PCr} = A_1\tau_1 \quad (2)$$

$$AC_{[La-]+EPOCfast} = E_{PCr} + E_{[La-]} \quad (3)$$

Where $\dot{V}O_{2(t)}$ corresponds to the oxygen uptake at time t, $\dot{V}O_{Rest}$ is the rest oxygen uptake, A_1 is the amplitude, and τ_1 is the constant time.

In addition, the oxidative metabolism contribution (E_{OXID}) was estimated considering the accumulated $\dot{V}O_2$ during the supramaximal effort using the trapezoidal method, excluding the rest values.

Statistical Analysis

The sample size was calculated (software G*Power) based on power analysis, taking into consideration the statistical power of 90%, α error probability of 0.05, and the effect size estimated from $AC_{[La-]+EPOCfast}$ mean and standard deviation differences in placebo and caffeine supplemented conditions (2), resulting in a minimum sample size of 13 participants. The results are presented as mean ± SD and 95% confidence interval (CI95%). Initially, the data were submitted to the Shapiro-Wilk test to verify data normality. The variables related to $AC_{[La-]+EPOCfast}$ in the caffeine and placebo conditions were compared using the paired t-test. The association was analyzed using the Pearson's product-moment correlation test. In all tests a level of significance of 5% was assumed. In addition, the data were analyzed qualitatively by magnitude-based inference and expressed as raw mean differences. The threshold values for

Cohen's d statistical power were considered as >0.2 (small), >0.5 (moderate), and >0.8 (large). The chances of a possible substantial benefit or harm were calculated [assuming the value of 0.2 multiplied by the between-subject deviation as the smallest worthwhile change (SWC)].

RESULTS

Table 2 displays the physiological responses at exhaustion in the GXT. All subjects reached the criteria to confirm $\dot{V}O_{2max}$ determination.

The 115% of i$\dot{V}O_{2max}$ was 335.2 ± 49.7 W (CI95% = 306.5 to 363.9 W). **Figure 2** shows in mean ± SD and individual values that the time to exhaustion (tlim) was higher (Δ% = +7.8%) in the caffeine condition (CI95% = 169.4 to 203.9 s) than in the placebo condition (CI95% = 158.7 to 187.9 s) (P = 0.006) **(Figure 2A),** with 10 out of 14 subjects with performance higher than SWC boundary (**Figure 2B**). Significant correlations were found between the conditions in tlim (r = 0.86; CI95% = 0.60 to 0.95; p = 0.00008).

(**Figures 3A,C,E**) shows in mean ± SD the individual values of the $AC_{[La-]+EPOCfast}$ values determined in the caffeine (CI95% = 3.56 to 4.44 L; CI95% = 51.5 to 57.7 mL·kg^{-1}; CI95% = 180.3 to 202.8 mL·kg^{-1}LM-LL) and placebo (CI95% = 3.57 to 4.54 L; CI95% = 51.9 to 58.5 mL·kg^{-1}; CI95% = 180.3 to 202.8 mL·kg^{-1} LM-LL) conditions. There were no differences between the $AC_{[La-]+EPOCfast}$ values expressed in absolute or relative terms (p > 0.708). In addition, when the data are analyzed individually (**Figures 3B,D,F**), 10 subjects from 14 participants modified the anaerobic capacity beyond upper/lower SWC limits (i.e., ∼4 subjects were "positive" responders and ∼7 subjects were "negative" responders), evidencing high inter-individual variability, while ∼3 subjects were "non-responders." Significant correlation was found only for the $AC_{[La-]+EPOCfast}$ expressed in absolute values (r = 0.74 and p = 0.002), but not when expressed in relative values (r = 0.12 and p = 0.68 for relative to body mass and r = 0.40 and p = 0.16 for relative to lean mass of lower limbs).

Table 3 shows the $\dot{V}O_2$, RPE, E_{OXID}, $E_{[La]}$, and E_{PCr} variables at exhaustion during the supramaximal efforts after caffeine or placebo supplementation. The exhaustion $\dot{V}O_2$ and RPE in both conditions were not statistically different and demonstrated significant correlations. Furthermore, the $E_{[La]}$ and E_{PCr} were not different and presented significant correlations (between conditions), while the E_{OXID} was higher in the caffeine than in the placebo condition.

DISCUSSION

The aim of the study was to verify the effects of acute caffeine supplementation on $AC_{[La-]+EPOCfast}$ in cycling. The main finding of this study was that acute caffeine supplementation improved the time to exhaustion during the supramaximal effort at 115% of i$\dot{V}O_{2max}$, but the $AC_{[La-]+EPOCfast}$ remained unaltered.

It has been shown that acute caffeine supplementation affects performance and the conventional MAOD estimate (3, 4). Bell et al. (3) found an improvement in the time to exhaustion at 125% of $\dot{V}O_2$ peak intensity and ∼7% increase in the MAOD value after supplementation with 5 mg·kg^{-1} of caffeine in untrained subjects. Corroborating these findings, Doherty (4) found an improvement in the MAOD values (∼10%) and time to exhaustion (∼14%) in untrained subjects with the same caffeine dosage. The explanation of these authors for the improvement in MAOD values was the greater mobilization of the glycolytic metabolism, leading

TABLE 2 | Values of heart rate (HR), respiratory exchange ratio (RER), peak blood lactate concentration ($[La^-]_{Peak}$), maximal oxygen uptake ($\dot{V}O_{2max}$), intensity associated with $\dot{V}O_{2max}$ (i$\dot{V}O_{2max}$), and total time obtained in graded exercise test (n = 14).

	HR (bpm)	RER	$[La^-]_{Peak}$ (mmol·L^{-1})	$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	i$\dot{V}O_{2max}$ (W)	Total time (min)
Mean ± SD	186.2 ± 8.3	1.21 ± 0.06	10.0 ± 1.9	52.8 ± 8.6	298.6 ± 48.4	14.5 ± 4.0
(CI95%)	(181.1 to 191.2)	(1.17 to 1.25)	(8.9 to 11.2)	(47.8 to 57.8)	(270.6 to 326.6)	(12.1 to 16.9)

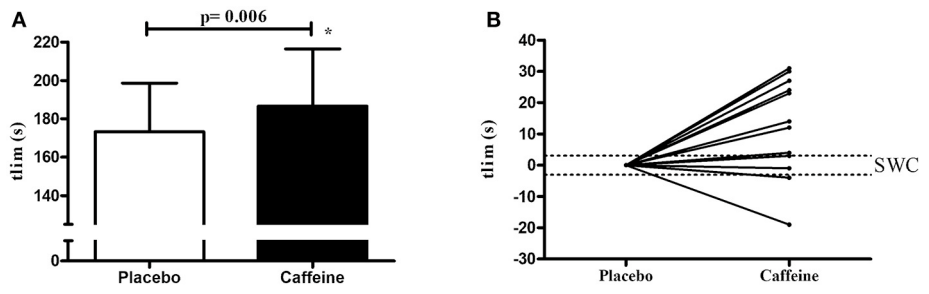

FIGURE 2 | Time to exhaustion (tlim) measured during supramaximal effort at 115% of i$\dot{V}O_{2max}$ under placebo and caffeine conditions. **(A)** shows the values in mean and standard deviation, while the figure **(B)** shows individual changes relative to placebo values and in relation to boundaries of smallest worthwhile change (SWC).

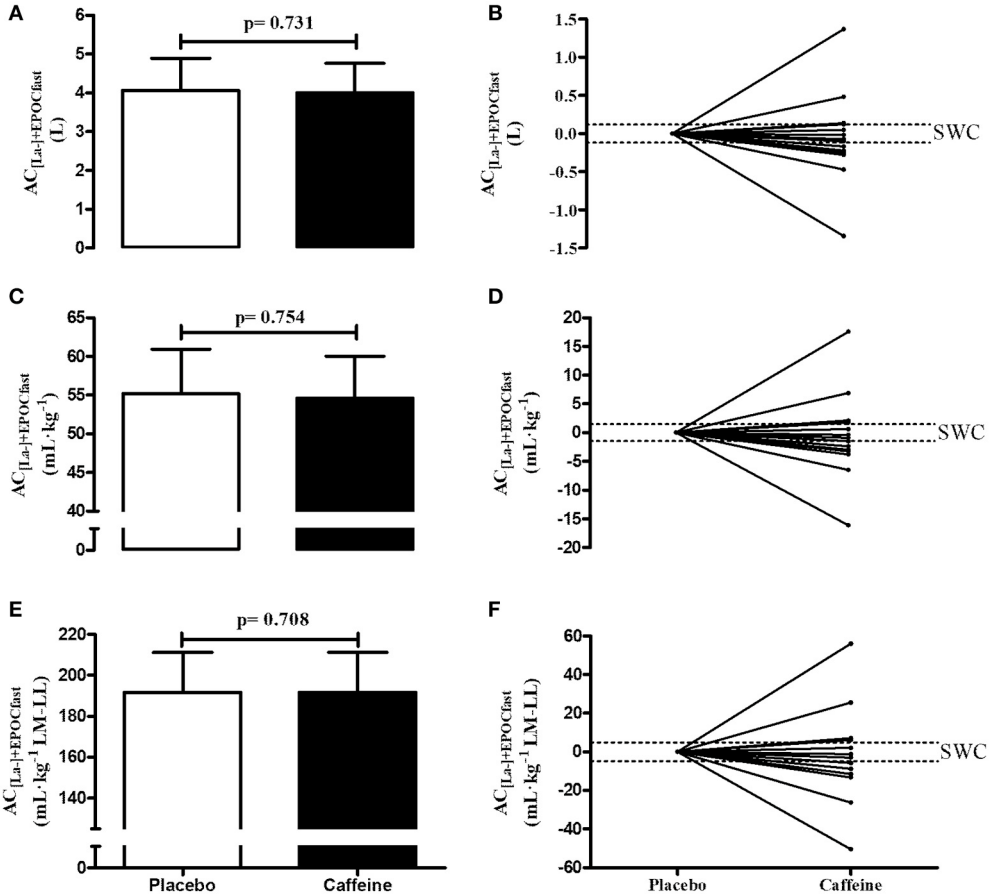

FIGURE 3 | $AC_{[La-]+EPOCfast}$ values in the caffeine and placebo conditions expressed in values absolute **(A,B)**, relative to body mass **(C,D)** and relative to lean mass of lower limbs **(E,F)** (n = 14). **(A,C,F)** show the values in mean and standard deviation, while the figures **(B,D,F)** show individual changes relative to placebo values and in relation to boundaries of smallest worthwhile change (SWC).

to high production and accumulation of lactate. However, Simmonds et al. (25) found that the iso-time accumulated $\dot{V}O_2$ and oxygen deficit during supramaximal efforts were similar with caffeine (5 mg·kg^{-1}) and placebo supplementation. In addition, these authors found no differences in the $\dot{V}O_2$ kinetics during the supramaximal efforts, demonstrating that the greater MAOD values in the caffeine than placebo condition were not related to changes in the relative contribution of the aerobic and anaerobic metabolisms, but were more related to increased time to exhaustion and a consequently greater deficit. In contrast to the results of these authors, in the present study we observed an improvement in time to exhaustion (**Table 3**), but not in $AC_{[La-]+EPOCfast}$ values after caffeine ingestion.

These differences between the results found in the literature and in the present study can possibly be explained by the methodological issues involved in determining the MAOD and $AC_{[La-]+EPOCfast}$. Medbø et al. (9) suggested that the supramaximal test to determine the MAOD should cause exhaustion in a minimum of 2 min due to the need to achieve the maximum capacity of energy production through anaerobic pathways. These authors demonstrated that MAOD increased with increasing time to exhaustion, reaching a plateau from 2 min on. In both the studies of Bell et al. (3) and Simmonds et al. (25) the mean values of time to exhaustion in the placebo condition (108.2 ± 8.9 s and 93.5 ± 24.1 s, respectively) were lower than the recommended time (i.e., 2–3 min) (26). Thus, this could have caused underestimation of MAOD values in the placebo condition and demonstrated a value closer to the "real" MAOD in the condition supplemented with caffeine due to the increased time to exhaustion. Considering the Simmonds et al. (25) findings that the on kinetics of $\dot{V}O_2$ are not altered with caffeine supplementation, the higher time to exhaustion causes an increase in the oxygen deficit. It is worth emphasizing that the $AC_{[La-]+EPOCfast}$ does not present this "limitation" as it is based on blood lactate and EPOC responses, and not directly on time to exhaustion. In addition, it has been shown that $AC_{[La-]+EPOCfast}$ remains unaltered at different supramaximal intensities (8).

Astorino et al. (1) found that acute supplementation with caffeine changed the magnitude of EPOC after a resistance training session. However, these authors analyzed the area under

TABLE 3 | Values of oxygen uptake ($\dot{V}O_2$) at exhaustion, rate of perceived exertion (RPE), and energetic contribution measured at 115% of $i\dot{V}O_{2max}$ after caffeine and placebo supplementation (n = 14).

	Placebo	Caffeine	p-value	Δ%	Pearson's r (CI95%)
$\dot{V}O_2$ Exhaustion ($mL{\cdot}kg^{-1}{\cdot}min^{-1}$)	51.2 ± 8.7 (46.2 to 56.2)	51.3 ± 8.5 (46.4 to 56.2)	0.753	± 0.4	0.98¥ (0.95 to 0.99)
RPE	18 ± 2 (17 to 19)	18 ± 2 (17 to 19)	1.000	-0.3	0.83¥ (0.53 to 0.95)
E_{OXID} (L)	7.44 ± 2.68 (5.90 to 8.99)	8.15 ± 2.96 (6.44 to 9.86)*	0.014	± 9.7	0.95¥ (0.84 to 0.98)
$E_{[La-]}$ (L)	2.53 ± 0.59 (2.19 to 2.87)	2.55 ± 0.51 (2.25 to 2.84)	0.834	± 2.5	0.82¥ (0.51 to 0.94)
$[La^-]_{Rest}$ ($mmol{\cdot}L^{-1}$)	1.1 ± 0.4 (0.9 to 1.3)	1.2 ± 0.4 (1.0 to 1.5)	0.444	± 16.1	0.17 (−0.39 to 0.64)
$[La^-]_{Peak}$ ($mmol{\cdot}L^{-1}$)	12.6 ± 1.5 (11.7 to 13.4)	12.8 ± 1.3 (12.0 to 13.9)	0.534	± 2.8	0.52 (−0.00 to 0.82)
$\Delta[La^-]$ ($mmol{\cdot}L^{-1}$)	11.4 ± 1.6 (10.5 to 12.3)	11.6 ± 1.3 (10.8 to 12.3)	0.736	± 2.4	0.49 (-0.05 to 0.81)
E_{PCr} (L)	1.53 ± 0.34 (1.33 to 1.73)	1.45 ± 0.42 (1.21 to 1.69)	0.448	-3.4	0.55* (0.02 to 0.82)
A_1 ($mL{\cdot}kg^{-1}{\cdot}min^{-1}$)	21.8 ± 4.4 (19.3 to 24.4)	21.1 ± 6.5 (17.3 to 24.8)	0.607	-2.8	0.54* (0.01 to 0.83)
τ_1 (min)	0.98 ± 0.21 (0.86 to 1.11)	1.03 ± 0.54 (0.72 to 1.34)	0.768	± 11.8	−0.17 (−0.64 to 0.39)

*Values are Mean ± SD (CI95%). *$P < 0.05$; ¥$P < 0.01$; E_{OXID} = net aerobic contribution; $E_{[La]}$ = glycolytic metabolism; $[La^-]$ = blood lactate; E_{PCr} = phosphagen metabolism; A_1 = amplitude 1; τ_1 = time constant 1.*

the EPOC curve, whereas in the present study we analyzed kinetics using a mathematical fitting to derive $EPOC_{FAST}$, which is thought to estimate the phosphagen metabolism pathway (6–8). Regardless of the EPOC method of analysis, the variables from the mathematical adjustment (i.e., A_1 and τ_1) used to determine the E_{PCr} in the present study were not statistically different. These findings do not corroborate Poli et al. (2) who found no significant differences in $AC_{[La-]+EPOCfast}$ determined in running after caffeine and placebo supplementation, but reported a lower τ_1 in the caffeine compared to placebo condition. The main argument of the authors for these differences in τ_1 is related to the greater exhaustion $\dot{V}O_2$ that allows a faster drop in $\dot{V}O_2$ after the effort. Nevertheless, in both studies the E_{PCr} was not significantly different in the caffeine and placebo conditions. Thus, considering that the effect of caffeine does not seem to be related to increased anaerobic energy supply and a possible effect on E_{PCr} in the caffeine condition would not be "wise," the E_{PCr} estimated by the $EPOC_{FAST}$ does not seem to be influenced by this ergogenic.

Mechanisms explaining the improvement in time to exhaustion after caffeine supplementation have been inconclusive. Possible explanations could be related to the effects of caffeine on stimulation of the central nervous system, an improvement in neuromuscular transmission, and in the contractility of muscle fiber (4). Simmonds et al. (25) suggested that caffeine can alleviate fatigue by maintaining electrolyte homeostasis at the beginning of the effort, maintaining the extracellular K^+ concentration and improving the action potential of the membrane, allowing muscle contraction and performance for a longer period before the onset of fatigue. However, in the present study the factors that could explain these mechanisms were not addressed.

However, the ergogenic effect of caffeine on performance is variable and seems to be associated to CYP1A2 polymorphism (27, 28), explaining the variable individuals effects with caffeine ingestion. In a recent study, Guest et al. (27) reported that some individuals are more responsible to caffeine ingestion than other 10 km cycling time-trial performance. The caffeine is metabolized by the CYP1A2 enzyme and these authors found that individual with CC genotype (i.e., homozygous slow metabolizers) decreased the performance compared to individuals with AA (fast metabolizers) and AC (heterozygous slow metabolizers) genotypes after 4 mg/kg intake of caffeine. These authors reported that in 101 participants 49% were AA, 43% AC, and 8% CC, and these range distributions can probably explain the inter-individual variations on performance and mainly on anaerobic capacity in the current study, with inter-individual range for anaerobic capacity change with caffeine compared with placebo was −24.5 to 34.2%. In addition, the **Figure 2B** shows that 10 of 14 subjects were positive responders to caffeine for performance while 2 were "non-responders" and 2 "negative" responders, evidencing that caffeine ingestion does not affect the anaerobic estimation.

A possible limitation of the study was the absence of $\dot{V}O_2$ kinetics analysis, which could partly explain the improved performance in the supramaximal effort and the unaltered $AC_{[La-]+EPOCfast}$ values. In addition, no analyses were performed related to the effects of caffeine on metabolite accumulation, or intracellular concentrations of K^+, or any analysis to explain the mechanisms related to the effects of caffeine on the stimulation of the central nervous system, which could contribute to clarifying the issues raised. Finally, it is important to report that the current study did not investigate genetic variation and its potential effects on the relationship between caffeine and the outcomes, and therefore this highlighted the individual responses with caffeine ingestion.

Therefore, we concluded that based on the overall comparison of mean values the acute caffeine supplementation improves the time to exhaustion in a supramaximal effort, although the E_{PCr}, $E_{[La]}$, and $AC_{[La-]+EPOCfast}$ remain unaltered. However, the effect of caffeine on the performance and anaerobic capacity depend on the individual.

PERSPECTIVE

The $AC_{[La-]+EPOCfast}$ method has been considered an advantageous method due to the reduced time taken to estimate anaerobic capacity (6–8). In addition, this method allows discrimination of the energetic equivalent of both the glycolytic and phosphagen pathways. For this reason, the effects of different ergogenic resources specifically on metabolic pathways have been investigated (16). Caffeine intake increases the time to exhaustion in the supramaximal effort and therefore possibly influences the determination of MAOD by the conventional method (25). However, $AC_{[La-]+EPOCfast}$ does not appear to be time-to-exhaustion dependent (8) and the present study investigated the possible effects of caffeine due to evidence of an impact on lactate concentrations (3) and on EPOC (1).This could influence the $AC_{[La-]+EPOCfast}$ values due to changes in the energy equivalents of the glycolytic and phosphagen pathways. However, the **Figures 2**, **3** which show individual data strongly suggest that individual responses to the caffeine vary quite markedly. Therefore, the findings of the present study showed that acute caffeine intake increases the time to exhaustion in the supramaximal effort, however does not alter the components of the E_{PCr} (i.e., amplitude and constant time of the mathematical adjustment), $E_{[La]}$ (i.e., lactate concentrations), and consequently the $AC_{[La-]+EPOCfast}$

AUTHOR CONTRIBUTIONS

WM participated in data acquisition, analyses and writing the manuscript. RB participated in manuscript writing. FN participated in the study design. RdP participated in data acquisition and analyses. AZ participated in the study design and writing the manuscript. All authors read and approved the final manuscript.

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REVIEW
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Time to Optimize Supplementation: Modifying Factors Influencing the Individual Responses to Extracellular Buffering Agents

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Blood alkalosis, as indicated by an increased blood bicarbonate concentration and pH, has been shown to be beneficial for exercise performance. Sodium bicarbonate, sodium citrate, and sodium or calcium lactate, can all result in increased circulating bicarbonate and have all independently been shown to improve exercise capacity and performance under various circumstances. Although there is considerable evidence demonstrating the efficacy of these supplements in several sports-specific situations, it is commonly acknowledged that their efficacy is equivocal, due to contrasting evidence. Herein, we discuss the physiological and environmental factors that may modify the effectiveness of these supplements including, (i) absolute changes in circulating bicarbonate; (ii) supplement timing, (iii) the exercise task performed, (iv) monocarboxylate transporter (MCT) activity; (v) training status, and (vi) associated side-effects. The aim of this narrative review is to highlight the factors which may modify the response to these supplements, so that individuals can use this information to attempt to optimize supplementation and allow the greatest possibility of an ergogenic effect.

Keywords: buffering agents, alkalosis, bicarbonate, citrate, lactate

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INTRODUCTION

Substances capable of increasing extracellular buffering capacity to combat exercise induced acidosis have been researched for almost a century. Sodium bicarbonate, sodium citrate, and sodium/calcium lactate can all result in alkalosis, indicated by an increase in blood pH and circulating bicarbonate, and all have independently been shown to improve exercise capacity and performance under various circumstances (1). Despite considerable evidence demonstrating these supplements to be effective in specific situations (2, 3), it is commonly acknowledged that their efficacy is equivocal due to contrasting evidence. Contradictory results from different investigations are commonly cited to highlight that the ergogenic effect from increased bicarbonate concentration is highly variable and is due to a variability in the individual responses. However, this may be oversimplifying the complex nature of individual responses (i.e., physiological and genetic

differences), whilst also overlooking experimental differences (loading and exercise protocols), sample populations (i.e., untrained; physically active; trained individuals), and design flaws (e.g., exercise unlikely to be affected by increased buffering capacity).

Recent developments in the way in which researchers analyze and interpret data is moving away from group means and toward more in-depth analysis of individuals responses (4). This is because we now know that there are several contributing factors that determine how individuals respond to supplementation, including their genetic composition, training status, habitual diet, and other circumstantial factors. Identification of any modifying factors that may alter the individual response to supplementation with extracellular buffering agents would provide vital information to clinicians, coaches, and athletes about the likelihood of gaining a positive or negative response. This would aid them in making fully informed decisions and optimize each individual's personal supplementation protocol. The aim of this review is to summarize the current evidence on the potential modifying factors underlying the individual response to supplementation with extracellular buffering agents.

THE ROLE OF PH IN SKELETAL MUSCLE FATIGUE

The ability of skeletal muscle tissue to maintain energy production and generate mechanical work is closely linked to performance in a variety of sports. The high turnover rate of skeletal muscle ATP that is seen during high-intensity exercise increases hydrogen ion (H^+) production, leading to muscle acidosis that is associated with performance loss (5). This is because an exercise-induced metabolic acidosis, characterized by a higher H^+ production rather than removal rate, can decrease energy substrate generation via glycolytic pathways by reducing the activity of key enzymes such as glycogen phosphorylase and phosphofructokinase (6). The H^+ also compete with calcium ions for the troponin binding site, directly hindering the muscle's contraction capacity (7). Oxidative phosphorylation can also be inhibited by acidosis (6) while resynthesis of phosphorylcreatine may also be compromised at low pH (8). Thus, a myoplasmic drop in pH drives an inability to produce the desired or required power output with a subsequent loss of exercise performance (9), although not all agree (10).

The body has several endogenous systems to control pH homeostasis; this balance is maintained by intracellular and extracellular buffers which can accept or release H^+ to prevent dramatic pH changes. In muscle, intracellular physicochemical buffers such as organic and inorganic phosphates, bicarbonate anions, and histidine containing dipeptides, are the primary mediators of pH homeostasis. There is also an active and passive transport of H^+ out of the muscle into the blood mediated by transport systems; during intense activity, H^+ efflux is mainly mediated by the lactate-proton transporters, namely monocarboxylate transporter 1 (MCT1) and 4 (MCT4) (11). Thereafter, the H^+ are buffered by the circulating anion bicarbonate (HCO_3^-), which forms carbonic acid, a weak acid. These endogenous buffering systems are well-regulated and highly efficient under normal physiological conditions (12). However, these systems can be quickly overwhelmed by the accumulation of H^+ during exercise, particularly when the intensity is high. Thus, increasing the buffering contribution of one or more of these systems is a feasible way to improve control of systemic pH changes and maintain exercise performance.

EXTRACELLULAR BUFFERING AGENTS

Several buffering agents are employed as ergogenic supplements, including sodium bicarbonate (SB), sodium citrate (SC), sodium lactate (SL), and calcium lactate (CL); the independent mechanisms through which they increase circulating bicarbonate will not be discussed here (for review see Lancha Junior et al. (1)). Nonetheless, all substances are ingested with the same focus: to increase the extracellular concentration of bicarbonate, increasing H^+ efflux out of the working muscle, thereby contributing to muscle acid-base balance during exercise, which may lead to an improved performance.

The ability of increased circulating bicarbonate to improve exercise capacity and performance has been extensively studied. It is widely acknowledged that the most effective extracellular buffer is SB, with numerous narrative reviews (13, 14) and meta-analyses (2, 3, 15, 16) demonstrating its efficacy. Meta-analytic data have suggested SC supplementation to be ineffective for performance (2), while limited data on SL and CL mean that no meta-analytical data on their efficacy currently exists. Despite this, supplementation with SC, SL, and CL has been shown to improve exercise capacity and performance on numerous independent occasions. Contrasting results in study outcomes following ingestion of these supplements is often cited as evidence that the response to these buffering supplements is variable. Such generalized statements do not consider the numerous contributing and modifying factors that influence the response to supplementation and do not allow any single individual to identify whether they are likely to benefit from supplementation or not. Thus, it is of importance to determine the factors that may contribute to an individual's response to these supplements.

POTENTIAL INFLUENCING FACTORS ON THE EFFICACY OF EXTRACELLULAR BUFFERS TO IMPROVE EXERCISE CAPACITY AND PERFORMANCE

Increases in Circulating Bicarbonate

To gain a competitive advantage from increased extracellular buffering capacity, there needs to be an increase in circulating bicarbonate following supplementation. Theoretically, any increase in bicarbonate would lead to a corresponding increase in buffering capacity; what the minimal increase necessary is to elicit performance gains is currently unknown. Carr et al. (17) have suggested that a +5 $mmol \cdot L^{-1}$ increase from baseline levels is required to have a potential ergogenic benefit in exercise performance, while a +6 $mmol \cdot L^{-1}$ increase leads

to almost certain ergogenic benefits. Despite this, no study to date has directly investigated the minimal increase necessary for performance gains or linked individual increases to change in performance. Saunders et al. (18) determined whether there were any correlations between blood values (bicarbonate, pH, base excess) and exercise capacity. Surprisingly, we did not show any relationship between the magnitude of change in circulating bicarbonate (or any other measure) and subsequent changes in exercise capacity.

Jones et al. (19) showed that no individual's maximal increases in circulating bicarbonate with a 0.1 $g \cdot kg^{-1}$ BM dose reached a +5 $mmol \cdot L^{-1}$ increase, which could explain why this dose appears to be ineffective for exercise performance (20). A 0.2 $g \cdot kg^{-1}$ BM dose, however, was effective at reaching this threshold in all individuals (19), although the mean increase 60 min post-ingestion was $< +5$ $mmol \cdot L^{-1}$. Nonetheless, it is possible that 60 min post-ingestion could coincide with a sufficient increase (i.e., $> +5$ $mmol \cdot L^{-1}$) in bicarbonate for some individuals. This could partially explain why a 0.2 $g \cdot kg^{-1}$ dose appears to be effective in some (20, 21), but not all (22–24), studies when exercise was performed 1 h post-supplementation. The time to reach this suggested minimal threshold varies between individuals (19), meaning it is possible that a higher proportion of individuals had not attained sufficient alkalosis in the studies showing no effect than those showing an effect, contributing to the group effects shown.

A 0.3 $g \cdot kg^{-1}$ BM dose of SB is the most commonly employed in the literature and appears to stem from the work of McNaughton who showed this to be most effective compared to lower and higher doses (20). Mean increases in circulating bicarbonate prior to exercise following a 0.3 $g \cdot kg^{-1}$ BM dose of SB is approximately +5–6 $mmol \cdot L^{-1}$ (25–27), and thus should be sufficient to improve exercise capacity and performance (19). Indeed, numerous studies have shown exercise gains with SB at this dose (28–38), though similarly, substantial data exist showing no effect following supplementation (26, 27, 34, 39–43). This may, in part, be explained by the absolute increases in blood bicarbonate shown prior to exercise, which appears to differ substantially between studies (**Figure 1**). Certainly, this visual demonstrates the importance in mean increases in circulating bicarbonate prior to subsequent exercise performance, although it must be acknowledged that several further contributing factors may account for this variation including exercise models not limited by H^+ accumulation, genotype, associated-side effects, and individual variation in the response to supplementation.

Few studies have investigated the effect of repeated supplementation in the same individuals using the same exercise protocol. We previously showed that SB supplementation in the same individual produced consistent blood bicarbonate responses, but this only translated into a mean improvement in one of the four sessions when SB was ingested (27). Interestingly, only one individual improved on all four occasions and nine others on at least one occasion, suggesting that subsequent exercise performance is more variable than blood responses. Nonetheless, although mean increases in circulating bicarbonate were approximately +6 $mmol \cdot L^{-1}$ during each session, not all individuals may have achieved a sufficient increase in circulating bicarbonate on every occasion. Additionally, inconsistencies may also be due to the training status of the participants who were recreationally active; trained individuals are likely to perform more consistently with lower variation between tests and thus may be better able to consistently take advantage of increased buffering capacity. Indeed, Carr et al. (41) showed 2,000 m rowing performance was reliable following repeated acute (0.3 $g \cdot kg^{-1}$ BM) and chronic (0.5 $g \cdot kg^{-1} \cdot day^{-1}$ BM for 3 days) supplementation in well-trained rowers, although performance was not improved. Trained cyclists and triathletes did consistently improve their cycling tolerance when administered SB on repeated occasions (35). Thus, it is possible that a proportion of the inconsistency in the response to SB (and potentially SC, CL, and SL) may be attributed to the training status of the individual and it would be of interest to determine the within-participant repeatability following supplementation using trained individuals.

Like the 0.3 $g \cdot kg^{-1}$ BM dose of SB, the most commonly employed dose of SC is 0.5 $g \cdot kg^{-1}$ BM and can be attributed to the pioneering work of McNaughton (54). Significant increases in bicarbonate were shown 90 min following a dose as low as 0.1 $g \cdot kg^{-1}$ BM, but since circulating bicarbonate increased in a linear fashion, and the greatest amount of work completed during a 60-s maximal exercise test was following a 0.5 $g \cdot kg^{-1}$ BM dose, this was considered the most effective. And yet, despite impressive increases in blood bicarbonate following SC supplementation, numerous studies have shown no effect on exercise capacity or performance (55–57). In fact, meta-analytic data showed an unclear effect of SC on exercise, with pre-exercise alkalosis associated with a small but unclear effect on performance (2). Indeed, despite clear increases in blood bicarbonate, several studies have shown no positive effect on subsequent exercise capacity or performance theoretically limited by acidosis (**Figure 2**). Aside from other confounding factors, discussed herein that may have modified the ergogenic effect of supplementation with SC, these increases may be counteracted by some other physiological response which inhibits the ergogenic effect of increased buffering capacity.

Increases in blood bicarbonate following ingestion of calcium or sodium lactate appear less obvious. Several studies have shown increases of the magnitude of $\sim$+3 $mmol \cdot L^{-1}$ (67, 68) while further studies showed no increase in circulating bicarbonate following acute (26) and chronic (69) supplementation, which explains the lack of an ergogenic effect in these studies. The +3 $mmol \cdot L^{-1}$ increase shown by Morris et al. (68) was sufficient to improve exercise tolerance by 17% during a cycling capacity test. Similarly, Morris et al. (70) showed improved high-intensity exercise capacity with mean bicarbonate increases of only +2.5 and +2.6 $mmol \cdot L^{-1}$ following supplementation of 120 and 300 $mg \cdot kg^{-1}$ BM of CL. Lactate supplementation improved running performance by a modest 1.7% (71), although changes in bicarbonate in this study cannot be determined since blood was only sampled at a solitary timepoint immediately prior to exercise. Low (150 $mg \cdot kg^{-1}$ BM) and high (300 $mg \cdot kg^{-1}$ BM) doses of CL lead to only moderate increases of +2 $mmol \cdot L^{-1}$ of bicarbonate, with no subsequent effect on repeated-bout high-intensity exercise (72). Certainly, the modest increases

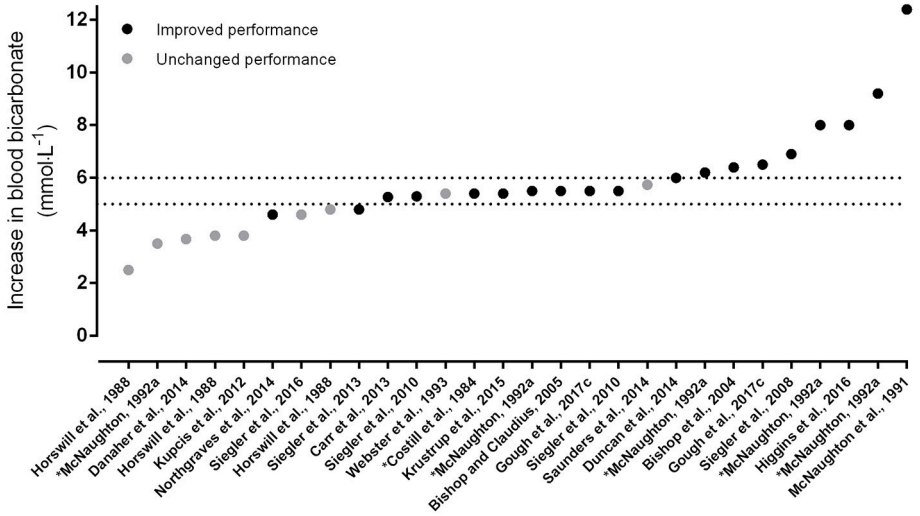

FIGURE 1 | Increases in blood bicarbonate from baseline following acute supplementation with sodium bicarbonate, in order of magnitude of change. Data points indicate whether exercise performance was improved with supplementation (dark circles) or not (light circles). The dotted lines indicate the thresholds for the zone of a potential ergogenic effect (+5 mmol·L^{-1}) and the zone of an almost certain ergogenic effect (+6 mmol·L^{-1}); taken from Carr et al. (2) and Jones et al. (19). *Denotes data estimated from graphs using specialized software (18, 20, 21, 23, 26, 29, 30, 33, 38, 42–53).

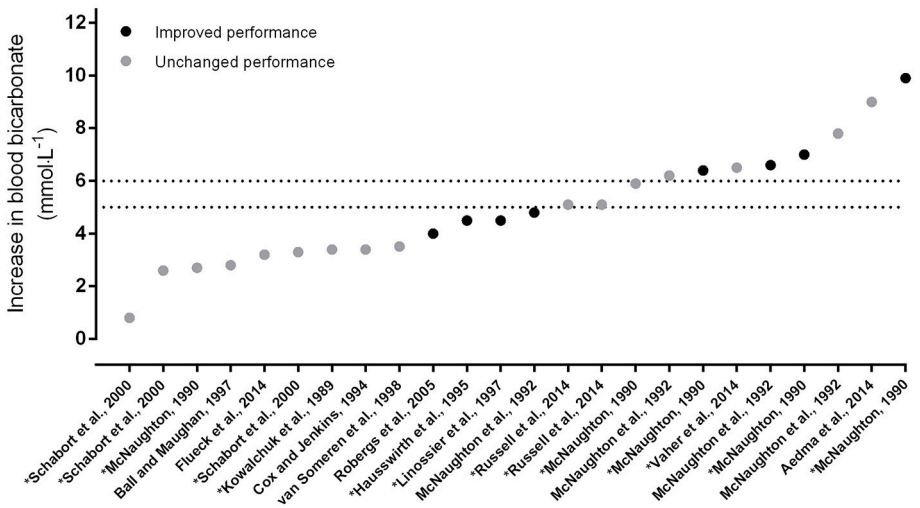

FIGURE 2 | Increases in blood bicarbonate from baseline following acute supplementation with sodium citrate, in order of magnitude of change. Data points indicate whether exercise performance was improved with supplementation (dark circles) or not (light circles). The dotted lines indicate the thresholds for the zone of a potential ergogenic effect (+5 mmol·L^{-1}) and the zone of an almost certain ergogenic effect (+6 mmol·L^{-1}); taken from Carr et al. (2) and Jones et al. (19). *Denotes data estimated from graphs using specialized software (24), (54–66).

in blood bicarbonate with lactate supplementation appear to be the primary factor behind the lack of an ergogenic effect shown in some, but not all, studies with lactate supplementation (**Figure 3**). Certainly, the individual range of bicarbonate increases could have contributed to this variability as, for example, the range in increases with low and high doses of calcium was between +0.4 and +6.7 mmol·L^{-1} and changes in bicarbonate were positively associated with the improvements in exercise capacity (70). More studies should relate the individual increases in circulating bicarbonate to changes in performance to provide insight into this relationship.

It is currently unclear what the minimal increase in circulating bicarbonate necessary is to result in an improvement in exercise performance. This has been suggested to be of the order of +5 mmol·L^{-1} (17), although studies have shown mean increases as little as +2.5 mmol·L^{-1} are sufficient to improve exercise tolerance (70), while +2 mmol·L^{-1} generally do not (72). Further research should investigate the smallest worthwhile

change in blood bicarbonate that can consistently lead to exercise gains.

Ingestion Timing

Siegler et al. (25) showed that increases in circulating bicarbonate and absolute concentration are similar at 60, 120, and 180 min post-ingestion of 0.3 g·kg^{-1} BM SB. Since performance during their repeated sprint protocol was not different when performed at these different post-ingestion timepoints, it could be assumed that precise timing of supplementation is of little importance to obtain performance gains. Nonetheless, recent interest has focused on the peak changes in circulating bicarbonate, with theory suggesting that maximal increases in blood bicarbonate will lead to maximal performance benefits, or at least the greatest chance of improvements. Most studies have employed a standardized supplementation protocol at a uniform timepoint. Although the data of Siegler et al. (25) showed that mean changes in circulating bicarbonate did not differ between timepoints, it is unlikely that all individuals performed exercise at a point which coincided with peak alkalosis.

A recent spate of investigations reporting the time course changes in blood markers following acute supplementation with SB (19, 73, 74), SC (75), and lactate (26, 72) suggest that a uniform supplementation timepoint is unlikely to be optimal for all individuals. The overwhelming conclusion that can be drawn from these independent investigations is that the time-to-peak bicarbonate concentration differs drastically between individuals, as does the absolute maximal change. Jones et al. (19) showed that peak bicarbonate concentration occurred between 30 and 150, 40 and 165, and 75 and 180 min following 0.1, 0.2, and 0.3 g·kg^{-1} BM of SB. Another study showed one individual who peaked only 10 min following supplementation of 0.3 g·kg^{-1} BM of SB (76). Absolute maximal changes in bicarbonate following SB ingestion varies greatly between individuals (**Table 1**), which could be a contributing factor to why some individuals respond more than others. Urwin et al. (75) showed that peak bicarbonate occurred much later than the commonly employed time given for supplementation with SC; indeed, it has recently been suggested that this may be a key factor contributing to the unclear effect shown with SC (77). Thus, if the greatest likelihood of an ergogenic effect occurs at the moment of peak circulating bicarbonate, then it is of great importance to determine individual time-to-peak bicarbonate following supplementation.

Determination of the time-to-peak bicarbonate concentration is only worthwhile for repeat ingestion if subsequent blood responses are reproducible. The consistency in blood responses following supplementation with extracellular buffers has only been determined with SB. Although both blood pH and bicarbonate responses showed good reliability following repeated supplementation with both 0.2 and 0.3 g·kg^{-1} BM doses of SB, blood bicarbonate responses were more reproducible, suggesting any individualized ingestion strategy should be based upon the time-to-peak bicarbonate (73). Nonetheless, although no statistical differences were shown in the time-to-peak, or the absolute maximal changes, it currently remains unclear whether minor changes in circulating bicarbonate significantly influence changes in exercise performance.

Two studies have investigated the effect of peak circulating bicarbonate concentration on exercise performance, both following SB supplementation. Overall repeated sprint performance was significantly improved with 0.3 g·kg^{-1} BM of SB compared to placebo and control (76). More recently it has been shown that 4-km cycling time-trial performance could be improved following both 0.2 and 0.3 g·kg^{-1} BM of SB when individualized to the time-to-peak blood bicarbonate

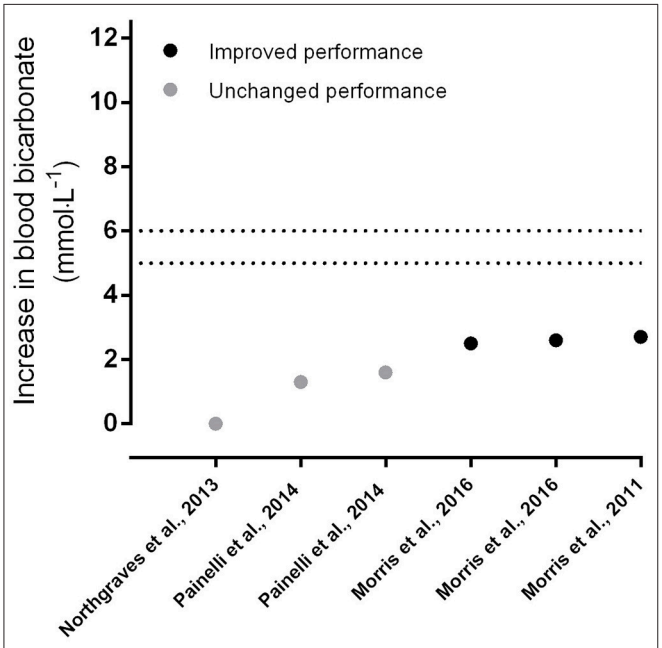

FIGURE 3 | Increases in blood bicarbonate from baseline following acute supplementation with calcium or sodium lactate, in order of magnitude of change. Data points indicate whether exercise performance was improved with supplementation (dark circles) or not (light circles). The dotted lines indicate the thresholds for the zone of a potential ergogenic effect (+5 mmol·L^{-1}) and the zone of an almost certain ergogenic effect (+6 mmol·L^{-1}); taken from Carr et al. (2) and Jones et al. (19). *Denotes data estimated from graphs using specialized software (26, 68, 70, 72).

TABLE 1 | Minimum (Min), maximum (Max), and mean time-to-peak and absolute maximal increases in blood bicarbonate concentration following supplementation with 0.1, 0.2, and 0.3 g·kg^{-1} BM sodium bicarbonate (SB) in Jones et al. (19).

Dose	Blood bicarbonate					
SB (g·kg^{-1} BM)	Time-to-peak (min)			Absolute change (mmol·L^{-1})		
	Min	Max	Mean	Min	Max	Mean
0.1	30	150	78 ± 34$^{*\Delta}$	+2.0	+5.0	+3.6 ± 0.8*
0.2	40	165	98 ± 32*	+5.1	+8.1	+6.1 ± 0.9*
0.3	75	180	123 ± 36	+6.0	+12.3	+8.2 ± 1.4

*$P < 0.05$ from 0.3 g·kg^{-1}BM; $^{\Delta}P < 0.05$ from 0.2 g·kg^{-1} BM.

(48). These findings are interesting considering two previous investigations showed no effect of SB supplementation on 4-km time-trial performance following a 0.3 $g \cdot kg^{-1}$ BM dose. Discrepancies could be due to timing and subsequent increases in circulating bicarbonate. Mean bicarbonate increases in Gough et al. (48) were $+6.5 \pm 1.3$ $mmol \cdot L^{-1}$, which is higher than the approximate $+3$ $mmol \cdot L^{-1}$ (78) and $+5$ $mmol \cdot L^{-1}$ (79) shown 150 and 100 min following supplement ingestion in the other studies. Despite these initial positive results, no data exist directly comparing exercise performed at the moment of peak circulating bicarbonate to a standardized time (e.g., 60 min) following supplementation in the same participants. As it stands, it cannot currently be concluded that exercise performed at peak bicarbonate concentrations elicits greater exercise improvements than normally seen with standardized ingestion prior to exercise.

Interestingly, recent data with SB show that determining time-to-peak blood bicarbonate concentration may allow a lower dose to be employed since maximal increases with 0.2 $g \cdot kg^{-1}$ BM were similar to those seen with 0.3 $g \cdot kg^{-1}$ BM, while 4-km time-trial performance was not different between these two doses (80). The absolute difference in maximal bicarbonate increases between these doses was ~ 1 $mmol \cdot L^{-1}$, suggesting that such a difference may not be meaningful. Since peak bicarbonate is only assessed by the absolute maximal value, and not via some statistical inference, it is possible that minor fluctuations in the actual bicarbonate concentration following large increases may not significantly influence subsequent exercise further, although work remains to be done to determine the smallest worthwhile change in blood bicarbonate that would influence exercise performance.

Exercise Task

An increase in buffering capacity via increases in circulating bicarbonate can lead to improvements in exercise capacity and performance, and the magnitude of this increase in buffering capacity appears to influence the likelihood of an effect, although even following substantial increases not all research is unanimous. These equivocal findings may also be due to the exercise protocols employed. If improvements are to be gained from increases in buffering capacity, exercise must be limited by increases in H^+ accumulation. Certainly, it has been suggested that short-duration exercise (in this case a 30 s cycle sprint) is unaffected by changes in muscle pH (81), while more endurance based activities rely increasingly on aerobic energy sources with no further increases in muscle acidosis. Therefore, improvements following supplementation with SB, SC, and CL and SL, are likely confined to specific exercise tasks which are significantly influenced by changes in acid-base balance. In fact, beta-alanine supplementation, which increases intracellular buffering capacity via increased muscle carnosine content, has been shown to be most effective during high-intensity exercise of 30 s to 10 min in duration, while longer and shorter duration exercise was unaffected (82). Since the mechanism of action of increased circulating bicarbonate is like that of beta-alanine supplementation (i.e., increased buffering capacity), comparable results would be expected.

Indeed, research appears to suggest exactly this. Despite similar increases in circulating bicarbonate following a 0.3 $g \cdot kg^{-1}$ BM dose of SB, exercise lasting 10 and 30 s in duration was unaffected, while exercise 120 and 240 s in duration was significantly improved (83). Identical results were shown following supplementation with 0.5 $g \cdot kg^{-1}$ BM of SC (63). Although some evidence suggests that buffering agents may be of benefit to longer duration exercise (36, 84), most studies report no improvements in continuous endurance exercise following supplementation (26, 57, 58, 85, 86). Multiple-bout high-intensity exercise, which has been shown to result in greater muscle acidosis than continuous supra-maximal exercise (87, 88), appears particularly susceptible to improvements with these supplements (21, 28–30, 38, 76, 89). Interestingly, Higgins et al. (34) showed that SB improved exercise capacity at 100% peak mean minute power, but not at 110 or 120%, despite the duration of all these exercise tasks being within the timeframe where an effect may be most likely expected (82).

Taken collectively, these results suggest that the likelihood of gaining a competitive edge from increased bicarbonate may be, in part, dependent on the exercise performed; the duration and intensity of the exercise appear to be key factors in this. Thus, the exercise task performed will be a determining factor in whether supplementation will be beneficial and cannot be overlooked; exercise gains with these substances should only be expected during exercise limited by the accumulation of H^+. Individuals should base their decision to supplement on their own exercise demands and the likelihood of gaining a worthwhile improvement therein. These are likely to be continuous high-intensity exercise tasks, such as 4 km cycling, 100 and 200 m swimming, and 2,000 m rowing, or repeated high-intensity activities such as those performed during team sports (e.g., football, hockey, basketball, etc).

Hydrogen Ion Transporters

The H^+ that accumulate in muscle throughout high-intensity exercise are predominantly removed by MCT1 and MCT4, through co-transport with lactate in a 1:1 ratio (11, 90). The sodium/hydrogen transport system (NHE) may also contribute to this process (91), although its relative contribution during exercise has been suggested to be minimal (90, 92). It is widely acknowledged that increased blood bicarbonate increases the activity of these MCT transporters, increasing the efflux of H^+ out of the muscle and reducing muscle acidosis (93). Surprisingly, no study to date has directly measured the effect of increased circulating bicarbonate via supplementation on the activity of these transporters in humans. It is currently unclear whether the relationship between the increased bicarbonate and the activity of these transporters is intrinsically associated.

Lactate transport has been shown to be elevated in athletes (94). This makes sense since training interventions have been shown to increase the abundance and activity of MCT transporters (95, 96). Furthermore, lactate transport capacity (and subsequently H^+ transport) was related to the occurrence of type I muscle fibers (97). MCT1 and MCT4 are both expressed in human skeletal muscle, although MCT1 is more prevalent in type I fibers and MCT4 in type II fibers (98). The higher affinity of

MCT4 (Km = 28–34 mM) compared to MCT1 (Km = 4–6 mM) for lactate, in addition to its higher prevalence in glycolytic fibers, suggests that MCT4 may be more important for efflux out of the working muscle (99). Hence, it could be suggested that any differences in training status or muscle fiber type predominance (i.e., trained sprinters vs. untrained individuals) may modify the response to supplementation with extracellular buffering agents. Indeed, this could, in part, explain some of the variation in responses between trained and non-trained individuals; a meta-analysis showed that the effect of SB supplementation on exercise was significantly lower for specifically trained compared to recreationally trained individuals (16). However, the exact mechanisms for this reduced effect has not been investigated and thus, suggestion that this may be due to differences in MCT transporter activity is currently highly speculative and warrants further investigation.

Polymorphisms in the MCT transporters may also influence an individual's response to supplementation. A single-nucleotide polymorphism in the gene coding for MCT1, for example, can influence the lactate/H^+ co-transport across the sarcolemma (100). The T allele carriers have been shown to have a reduced MCT1 lactate transport (101, 102), TT homozygotes reach the ventilatory threshold at higher speeds (103), while T allele frequency has been shown to differ between athletes of different modalities (100, 104). Despite this, the effect of this polymorphism on high-intensity exercise performance, with or without supplementation, is currently unclear. Although genetic variations of the MCT4 gene exist (105), to our knowledge, no study has investigated the relevance of any polymorphisms on lactate/H^+ flux and its effect on exercise performance. It is apparent that polymorphisms in the genes encoding for MCT1 and MCT4 could alter their activity during high-intensity activity; it could thus be implied that genetic differences may also modify the individual response to supplementation with buffering agents, although no data currently exist. It is essential that more research on genotypic differences in MCT transporters and response to supplementation is undertaken before any recommendations can be made.

Associated Side-Effects

An evident moderator of the efficacy of buffering agents to improve exercise capacity and performance, is their associated side-effects. The occurrence of gastrointestinal (GI) discomfort with these supplements is common, with stomach cramps, nausea, vomiting, and diarrhea among some of the most frequent complaints with SB and SC (17, 75), although the only reported side-effects with lactate supplementation was increased incidence of belching and flatulence (72). This has obvious implications for athletes considering supplementing during competition and is likely a contributing factor to why incidence of supplementation with such agents is low (106).

Following ingestion of SB, it is dissociated in the stomach acid to form sodium (Na^+) and bicarbonate, much of the latter of which is swiftly neutralized by H^+, thereby producing carbon dioxide [CO_2] (107). The production of CO_2 in the stomach may cause gastric discomfort with symptoms including bloating and abdominal pain; nausea and vomiting are other commonly reported side effects (17). A contributing factor to the intensity of any side-effects with SB is certainly dose, as McNaughton et al. (20) reported increasing discomfort with increasing doses above 0.3 g·kg^{-1} BM, with no concomitant increases in performance. The typical 0.3 g·kg^{-1} BM often results in reported side-effects which may moderate performance (2, 16), although whether they directly influence performance may be disputed. Indeed, Price and Simons (40) reported no association between GI discomfort and performance while data from our laboratory has shown on several occasions that exercise capacity may be modified by GI discomfort (18, 27). Specifically, all individuals reporting discomfort worsened performance, and reanalyzing data following the removal of these individuals modified the group level statistics, turning a non-significant result into a significant result. Thus, it is apparent that minimizing the discomfort associated with SB supplementation could increase the likelihood of a positive response, both at the individual and group level. A potential solution is to ingest SB in gastro-resistant capsules, avoiding neutralization in the stomach and the associated side-effects; this theory is currently being tested in our laboratory.

The timing of supplement ingestion may be a key modifiable component that can increase or decrease the likelihood of an ergogenic effect not only due to increased circulating bicarbonate, but also due to the associated side-effects. Siegler et al. (25) reported lower symptoms of GI discomfort 180 min following SB supplementation (compared to 60 and 120 min). The most intense symptoms with SC were 60–120 min following supplementation (75). Since supplements are commonly ingested at a standardized time, often 60 min prior to the commencement of exercise as in the case of SB, this suggests that most studies may have forced individuals to exercise at a time when they experience sufficient discomfort to negatively impact their performance. Thus, adopting an individual time-to-peak bicarbonate supplementation protocol would likely avoid performing exercise when associated side-effects are most intense. Although we have shown that side-effects with these supplements could modify their ergogenic effect, it cannot explain the lack of an effect in all individuals. Nonetheless, it is apparent that offsetting any discomfort with supplementation will avoid any possibility that side-effects will affect the exercise response.

OPTIMIZING SUPPLEMENTATION

It is becoming apparent that timing of ingestion, may be one of the key moderating factors to the ergogenic response to increased circulating bicarbonate. Coinciding exercise with the point at which bicarbonate peaks is likely to maximize the chance of exercise improvements since buffering capacity will be maximized. Importantly, early reports suggest that the time at which peak bicarbonate occurs with SB is repeatable within individuals (73); more information is needed regarding consistency in blood bicarbonate responses to other buffering agents (SC, SL, and CL). Furthermore, peak GI discomfort has

been shown to occur earlier than maximal increases in blood bicarbonate following SB (73, 80) and SC (75) supplementation. Reported side-effects with lactate supplementation are minimal and mean peak bicarbonate concentration occurred after 90 min although further information is necessary regarding the individual time course response. Altogether, data suggest that individualizing supplementation timing with these agents may provide the best opportunity of gaining an ergogenic effect by coinciding the moment of exercise with peak bicarbonate concentration following the selected dose.

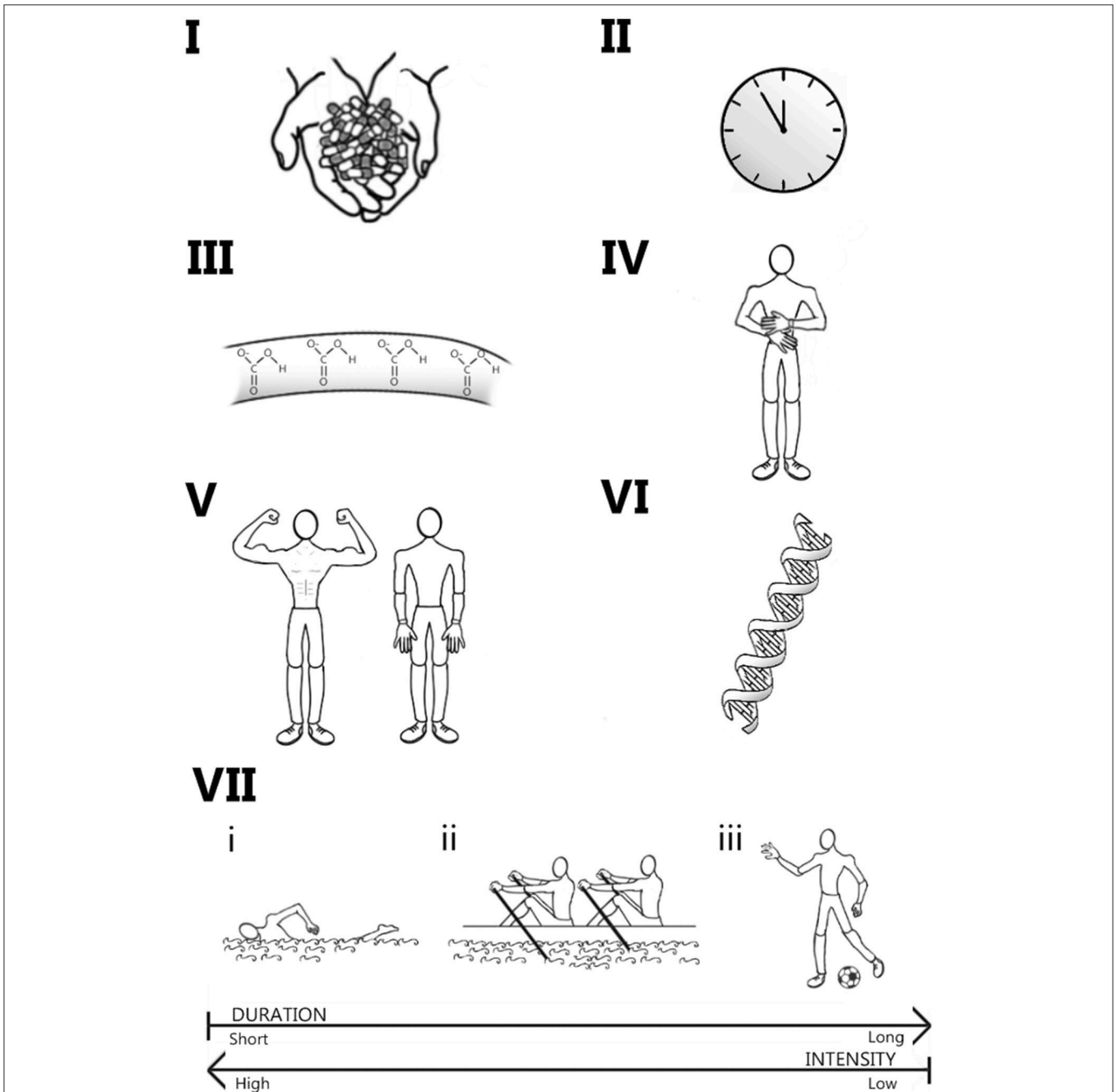

FIGURE 4 | Overview of the factors which may modify the response to supplementation with sodium bicarbonate, sodium citrate, and calcium and sodium lactate. The dose (Panel I) and timing of ingestion (Panel II) can influence both the increases in circulating bicarbonate (Panel III) and associated side-effects (Panel IV) at the moment of exercise, impacting upon the likelihood of a performance benefit. Training status (Panel V) and genetic make-up (Panel VI) of the individual may further modify this response. The chances of a positive response may also be modified by the duration and intensity of the exercise task being performed (Panel VII): short-duration continuous exercise, such as 100 and 200 m swimming (i) and 2,000 m rowing (ii), and prolonged intermittent activity, such as football (iii), may be particularly susceptible to improvements with these supplements.

In fact, supplement dose is another highly modifiable factor that could be adapted to optimize supplementation. Increasing doses of SB above 0.3 $g \cdot kg^{-1}$ BM results in increased incidence of GI discomfort, while 0.1 $g \cdot kg^{-1}$ BM appears insufficient to result in exercise improvements (20). Although 0.3 $g \cdot kg^{-1}$ BM has long been considered the optimal dose, recent evidence suggests that 0.2 $g \cdot kg^{-1}$ BM may be equally efficient at improving performance (80). Considering the lower incidence of GI discomfort with similar gains in bicarbonate, a 0.2 $g \cdot kg^{-1}$ BM ingested at a timepoint specific to the individual may be the most effective way of maximizing the chances of an ergogenic effect with SB. SC increased blood bicarbonate in a linear manner in doses increasing from 0.1 to 0.5 $g \cdot kg^{-1}$ BM (54); doses above 0.5 $g \cdot kg^{-1}$ BM of SC showed no further gains in blood bicarbonate but increased the incidence and severity of side-effects (75) suggesting 0.5 $g \cdot kg^{-1}$ BM of SC to be the optimal dose. Lactate supplementation results in modest increases in bicarbonate regardless of a 0.15 or a 0.3 $g \cdot kg^{-1}$ BM dose although no significant GI discomfort. Nonetheless, current doses of up to 0.3 $g \cdot kg^{-1}$ BM with CL or SL may be insufficient to improve exercise though further work is necessary to ascertain whether higher doses may be more beneficial.

PRACTICAL RECOMMENDATIONS

Individuals aiming to supplement with these substances should trial them outside of competition to see what works for them and adapt and optimize their individual supplementation strategies according to their own personal needs and responses. Since the time course response has been shown to be consistent (at least following SB ingestion), ingestion should occur at a time that means exercise begins when bicarbonate concentration has increased above this +5–6 $mmol \cdot L^{-1}$ threshold. Although there may be logistical difficulties, it would be necessary to determine the timeframe during which individuals increase their bicarbonate levels above +5–6 $mmol \cdot L^{-1}$, and side-effects are minimal, so that they can adapt their supplementation timing accordingly to optimize their chances of an effect. Individuals should be wary that any improvements in exercise are likely to be marginal, although this may be worthwhile for highly-trained or elite athletes. Furthermore, the type of exercise that individuals undertake will influence the likelihood of any benefit from increased bicarbonate. Short-duration high-intensity exercise, such as 4 km cycling, 2,000 m rowing, and 200 m swimming, are examples of activities likely to profit from increased buffering capacity and there is also evidence that individuals involved in prolonged intermittent exercise may benefit from supplementation. It appears the likelihood of obtaining an ergogenic effect is greater when SB is ingested compared to SC, SL, or CL, and it is the recommendation of these authors that 0.2–0.3 $g \cdot kg^{-1}$ BM SB is taken as the preferred supplement where possible to improve high-intensity exercise capacity and performance limited by acidosis.

CONCLUSIONS AND FUTURE DIRECTION

Sodium bicarbonate, sodium citrate and sodium/calcium lactate are supplements to increase circulating bicarbonate and have all independently been shown to improve exercise capacity and performance under various conditions. However, several factors may modify their ergogenic effects including supplement timing and dose, absolute changes in circulating bicarbonate, the exercise task being performed, MCT activity, training status, and associated side-effects (**Figure 4**). Emerging evidence suggests that, to maximize the chances of an ergogenic effect, supplementation should occur at a time point that results in exercise being performed at the moment of peak bicarbonate concentration, with minimal or no side-effects. More information is required regarding each supplement independently and their interaction with the individual, genotype, and the environment; these have been detailed throughout this article.

AUTHOR CONTRIBUTIONS

BS is responsible for the conception of the work. AH, PP, LO, and BS are responsible for the writing of the manuscript. LM reviewed the manuscript.

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REVIEW
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Sport Nutrigenomics: Personalized Nutrition for Athletic Performance

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An individual's dietary and supplement strategies can influence markedly their physical performance. Personalized nutrition in athletic populations aims to optimize health, body composition, and exercise performance by targeting dietary recommendations to an individual's genetic profile. Sport dietitians and nutritionists have long been adept at placing additional scrutiny on the one-size-fits-all general population dietary guidelines to accommodate various sporting populations. However, generic "one-size-fits-all" recommendations still remain. Genetic differences are known to impact absorption, metabolism, uptake, utilization and excretion of nutrients and food bioactives, which ultimately affects a number of metabolic pathways. Nutrigenomics and nutrigenetics are experimental approaches that use genomic information and genetic testing technologies to examine the role of individual genetic differences in modifying an athlete's response to nutrients and other food components. Although there have been few randomized, controlled trials examining the effects of genetic variation on performance in response to an ergogenic aid, there is a growing foundation of research linking gene-diet interactions on biomarkers of nutritional status, which impact exercise and sport performance. This foundation forms the basis from which the field of sport nutrigenomics continues to develop. We review the science of genetic modifiers of various dietary factors that impact an athlete's nutritional status, body composition and, ultimately athletic performance.

Keywords: nutrigenomics, nutrigenetics, personalized nutrition, athletic performance, genetic testing, sports nutrition, caffeine, ergogenic aids

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INTRODUCTION

Sport and exercise performance are significantly influenced by nutrition, yet individuals respond differently to the same foods, nutrients and supplements consumed. This holds true for a variety of ages, ethnicities, and level of skill, and whether the goal is optimizing physical activity for health and fitness or for high performance sport. The importance of a personalized sports nutrition plan was highlighted in the recent "Nutrition and Athletic Performance" Joint Position Statement by the American College of Sports Medicine, the Academy of Nutrition and Dietetics and the Dietitians of Canada, which states that "Nutrition plans need to be personalized to the individual athlete… and take into account specificity and uniqueness of responses to various strategies" (1). These strategies encompass overall dietary patterns, macronutrient ratios, micronutrient requirements, eating behaviors (e.g., nutrient timing), and the judicious use of supplements and ergogenic aids.

The paradigm shift, away from the one-size-fits-all group approach and toward personalization for the individual, is moving nutrigenomics research from basic science into practice. While it has long been recognized that genetics play an influential role in determining

how an athlete responds to foods and nutrients, the surge in research into gene-diet interactions over the past decade has provided a scientific basis for this hypothesis through various research initiatives and the corresponding increase in published studies. Genetic variants affect the way we absorb, metabolize, utilize and excrete nutrients, and gene-diet interactions that affect metabolic pathways relevant to health and performance are now widely recognized (2). Personal genetic testing can provide information that will guide recommendations for dietary choices that are more effective at the individual level than current dietary advice, which has been set by government agencies and other health and sport organizations. Disclosure of genetic information has also been shown to enhance motivation and behavior change and strengthen adherence to the dietary recommendations provided (2–6). Although athletes tend to exhibit higher levels of motivation in general (7), nutrition professionals still encounter significant barriers to behavior change when counseling athletes on the adoption of beneficial sports nutrition practices (8, 9). A recent systematic review found that when genetic information included actionable advice, individuals were more likely to change health behaviors, including their dietary choices and intakes (10).

The practical application of the scientific knowledge gained from research on health and performance is to enable athletes to utilize genetic test results for personalized nutrition in an actionable manner. The demand for genetic testing for personalized nutrition and associated performance outcomes by athletes and active individuals is growing, and there is an increased need for dietitian-nutritionists, fitness professionals, coaches, and other sports medicine practitioners to understand the current evidence in this developing field (11–14). The sport environment is dynamic, progressive, innovative, and extremely competitive. Providing athletes with individually tailored dietary and other performance-related information based on their DNA could yield a competitive edge. The growing body of science in nutrition and genetics is the foundational building block by which practitioners can help athletes reach their genetic potential through implementation of dietary and supplement strategies that are aligned to their genetic makeup (**Figure 1**). Scientific advancements along with increased interest in genetic testing have resulted in a necessary growth for professional support, where tools for proficient and knowledgeable nutrition counseling based on genetics are now more widely available. For example, the Dietitians of Canada now offer a course on "Nutrigenomics: Genetic testing for personalized nutrition" as part of their online Learning-on-Demand portal.

Personalized nutrition, based on an individual's genotype, is not a novel concept, and there are several examples of rare (e.g., phenylketonuria) and common (e.g., lactose intolerance) genetic variants that require specific dietary strategies to manage (15). Although genetic testing is well-established in the clinical setting, there is a growth in opportunities to improve health, wellness and sport performance in athletes through nutrition-focused genetic testing. In the ongoing battles against dangerous supplements (16) and unprecedented numbers of doping violations (17, 18), the sport science community is seeking novel, yet evidence-based, approaches for athletes to gain a competitive edge which are safe, effective and legal. Personalized nutrition is not limited to the identification of genetic variants. Genotype is one aspect of personal information that can be used to individualize dietary advice. An individual's genetic profile as it relates to diet should be used combination with other relevant information such as sex, age, anthropometrics, health status, family history, and socioeconomic status along with dietary preferences and the presence of food intolerances or allergies. Accompanying blood work is also useful to evaluate current nutrition status and for ongoing monitoring.

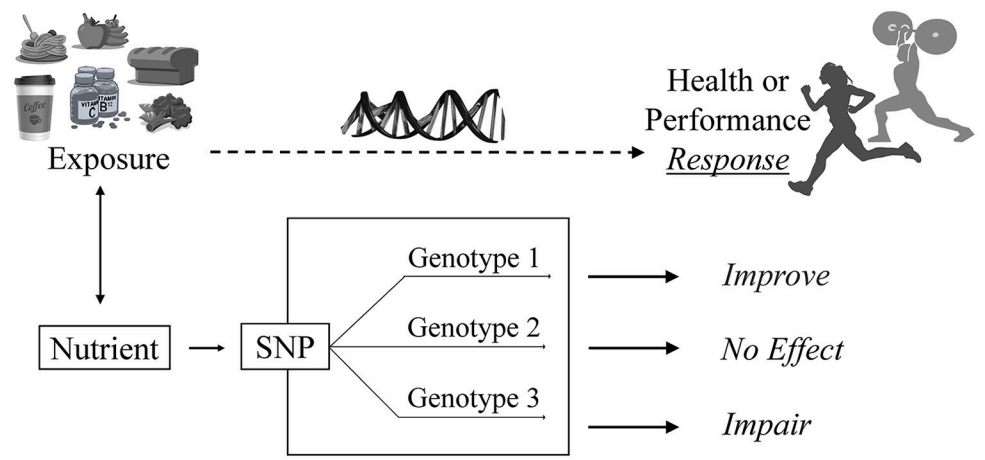

FIGURE 1 | The nutrigenomics approach to sport nutrition. An athlete is exposed to a food, beverage, nutrient or bioactive. A genetic variant such as a single nucleotide polymorphism (SNP) associated with that exposure modifiers the individual's requirement for or response to that exposure. Their unique response depends on their version of the gene or "genotype." For example, in the *CYP1A2* rs726551 SNP, individuals with the AA genotype (fast metabolizers) experience a positive or "improved" response (i.e., performance) to caffeine. Individuals with the *CYP1A2* AC or CC genotype experience no effect or impaired performance, respectively, from caffeine use (19).

Personalized dietary and supplement advice derived from genetic testing should be based on clear and defensible interpretations of relevant research studies. Traditional genome-wide association studies (GWAS) can be used to identify associations between genotypes and outcomes of interest such as blood levels of a micronutrient. However, the utility of such markers in providing actionable information on dietary advice is limited because it is not known what dietary intakes are required to counter effects of the genetic variant(s). For example, although a genetic variant that has been associated with low serum values of a vitamin is identified, a specific recommendation for intakes to prevent the risk of deficiency or to alleviate low levels of this micronutrient may remain undetermined. Such studies require the appropriate design that demonstrates how a genetic variant modifies the response to dietary intake on the outcome trait of interest and perhaps identify responders and non-responders. Genetic markers related to a performance trait, such as aerobic capacity or power, also provide little information on what factors could be used to improve the trait of interest.

With the exception of investigations exploring genetic variation and supplemental caffeine, which have been shown to modify endurance exercise outcomes (19, 20), there are few performance studies that have examined the role of genetics and other dietary factors on athletic outcomes. A gene-diet interaction may not be associated directly with a quantifiable performance outcome, such as increased aerobic capacity, speed or strength, but rather with intermediate biomarkers or phenotypes, such as body composition or circulating vitamin D levels, which are independent determinants of athletic performance, injury-risk and post-training recovery (1, 21–24). For example, it is well-known that low iron stores impact hemoglobin production which in turn decreases the oxygen carrying capacity of the blood, leading to a lack of oxygen to working muscles and resulting in impaired muscle contraction and aerobic endurance (21). As such, genetic markers that impact iron stores in response to intake can indirectly affect performance through the oxygen carrying capacity of hemoglobin (25, 26).

Sport Nutrigenomics Vs. Talent Identification and Exercise Prescription

In an effort to achieve specific sport goals, there is generally considerable overlap in the development of complementary training and dietary plans for athletes (27–29). However, it is essential to underscore the distinction between the strength of evidence supporting DNA-based advice for personalized nutrition vs. that for fitness programming. Despite the fervent interest and ubiquity of commercial genetic testing to assess and improve exercise or sport performance (30–32), it should be noted that there is a lack of evidence encompassing exercise prescription and talent identification, such as the ability to predict the likelihood for the next generation of Olympians (33, 34). Similarly, at this time there is insufficient evidence to recommended training protocols (strength or endurance) based on genotype or polygenic scores, that target specific fitness, weight loss or sport goals (35–38). The practical and ethical considerations of genetic testing for sports performance have also been described (39).

Some commercial genetic tests claim to use proprietary algorithmic approaches to prescribe training protocols based on evidence reported in peer-reviewed research (35). Although this may provide some initial supportive documentation for differing responses to training based on genotype, much larger sample sizes and improved methodologies are required, and should be pursued (36). The approach by which individuals are categorized as having an "endurance" or "power" advantage by genotype or being "responders" and "non-responders" to different training protocols, requires transparency and standardization across the field to avoid potential bias and to allow other researchers to replicate a study's methodology (37). Attempts to replicate studies to test training outcomes based on genotype require the use of the identical scoring systems and it appears that essential details of methods for grading of the strength of scientific evidence used in these scoring systems are not reported (35).

There is a considerable amount of ongoing research investigating individual variation in response to exercise training, however, sport and exercise genomics is still in its early stages and clinical or sport utility is lacking (36, 40–44). Mainstream testing for personalized training or exercise prescription based on genotype is not currently supported as a scientifically-sound approach, although it is likely to be a common and viably employed coaching tool within the next decade (35–37, 43, 44).

GENES ASSOCIATED WITH SPORT NUTRITION

The objective of this review is to examine the scientific evidence on specific nutrients and food bioactives whereby genetic variants appear to modify individual responses related to athlete health and athletic performance. Although many studies reviewed herein have not been studied in athletes exclusively, they have been carried out in healthy individuals. Accordingly, several studies outlined reflect optimal health, body composition and nutritional status, which for athletes, provides the foundation for athletic success. Genetic variation impacting response to various micro- and macronutrients, as well as bioactives such as caffeine, on performance-related traits will be reviewed (**Table 1**).

Caffeine

Caffeine, found naturally occurring in several plant species including coffee, tea, cocoa, and guarana, is widely used in sport as a performance enhancer or ergogenic aid often in the form of caffeinated tablets, gels or chews.

In the field of nutrigenomics, caffeine is the most widely researched compound with several randomized controlled trials investigating the modifying effects of genetic variation on athletic performance (19, 20, 45). Numerous studies have investigated the effect of supplemental caffeine on exercise performance, but there is considerable inter-individual variability in the magnitude of these effects (46–48), or in the lack of an effect (49, 50) when compared to placebo. These inter-individual differences

TABLE 1 | Summary of Genetic Variants that modify the association between various dietary factors and performance-related outcomes.

Gene (rs number)	Function	Dietary factor	Dietary sources	Performance-related outcome
CYP1A2 (rs762551)	Encodes CYP1A2 liver enzyme: metabolizes caffeine; identifies individuals as fast or slow metabolizers	Caffeine	Coffee, tea, soda, energy drinks, caffeine supplements	Cardiovascular health, endurance (21, 22, 57, 58)
ADORA2A (rs5751876)	Regulates myocardial oxygen demand; increases coronary circulation via vasodilation	Caffeine	Coffee, tea, soda, energy drinks, caffeine supplements	Vigilance when fatigued, sleep quality (49, 51–53)
BCMO1 (rs11645428)	Converts provitamin A carotenoids to Vitamin A	Vitamin A	Bluefin tuna, hard goat cheese, eggs, mackerel, carrots, sweet potato	Visuomotor skills and immunity (93, 95, 98–101)
MTHFR (rs1801133)	Produces the enzyme methylenetetrahydrofolate reductase, which is involved in the conversion of folic acid and folate into their biologically active form, L-methylfolate	Folate	Edamame, chicken liver, lentils, asparagus, black beans, kale, avocado	Megaloblastic anemia and hyperhomocysteinemia risk (112, 116–118)
HFE (rs1800562 and rs1799945)	Regulates intestinal iron uptake	Iron	Beef, chicken, fish, organ meats (heme iron); almonds, parsley, spinach (non-heme iron)	Hereditary hemochromatosis (130–132)
TMPRSS6 (rs4820268), TFR2 (rs7385804), TF (rs3811647)	Regulate the peptide hormone, hepcidin, which controls iron absorption	Iron	Beef, chicken, fish, organ meats (heme iron); almonds, parsley, spinach (non-heme iron)	Iron-deficiency anemia risk (24, 27, 120, 123–125)
FUT2 (rs602662)	Involved in vitamin B12 cell transport and absorption	Vitamin B12	Clams, oysters, herring, nutritional yeast, beef, salmon	Megaloblastic anemia and hyperhomocysteinemia (142)
GSTT1 (Ins/Del)	Plays a role in vitamin C utilization via glutathione S-transferase enzymes	Vitamin C	Red peppers, strawberries, pineapple, oranges, broccoli	Circulating ascorbic acid levels Mitigate exercise-induced ROS production (153, 155)
GC (rs2282679) and CYP2R1 (rs10741657)	GC encodes vitamin D-binding protein, involved in binding and transporting vitamin D to tissues; CYP2R1 encodes the enzyme vitamin D 25-hydroxylase involved in vitamin D activation	Vitamin D	Salmon, white fish, rainbow trout, halibut, milk	Circulating 25(OH)D levels impacting immunity, bone health, inflammation, strength training and recovery (1, 162, 164, 166, 168)
GC (rs7041 and rs4588)	GC encodes vitamin D-binding protein, involved in binding and transporting vitamin D to tissues; Vitamin D is required for calcium absorption	Calcium	Yogurt, milk, cheese, firm tofu, canned salmon (with bones), edamame	Bone/stress fracture risk Muscle contraction, nerve conduction, blood clotting (162, 164, 166, 168)
PEMT (rs12325817)	Involved in endogenous choline synthesis via the hepatic phosphatidylethanolamine *N*-methyltransferase pathway	Choline	Eggs, beef, poultry, fish, shrimp, broccoli, salmon	Muscle or liver damage, reduced neurotransmitters (174, 175, 185, 186)
MTHFD1 (rs2236225)	Encodes protein involved in trifunctional enzyme activities related to metabolic handling of choline and folate	Folate/Choline	Folate: Edamame, chicken liver, lentils, asparagus, blck beans, kale, avocado Choline: Eggs, beef, poultry, fish, shrimp, broccoli, salmon	Muscle or liver damage, reduced neurotransmitters (185, 186)
FTO (rs1558902/rs9939609)	Precise function undetermined; plays a role in metabolism and has been consistently linked to weight, BMI and body composition	Protein/SFA:PUFA	Protein: chicken, beef, tofu, salmon, cottage cheese, lentils, milk, Greek yogurt SFA: cheese, butter, red meat, baked goods PUFA: flaxseed oil, grape seed oil, sunflower oil	Optimizing body composition (190, 191)
TCF7L2 (rs7903146)	Involved in expression of body fat	Fat	Nuts/seeds, butter, oils, cheese, red meat, high-fat dairy	Optimizing body composition (192, 193)
PPARγ2 (rs1801282)	Regulates adipocyte differentiation	MUFA	Macadamia nuts, almond butter, peanut butter, olive oil, canola oil, sesame oil	Optimizing body composition (194)

appear to be partly due, to variation in genes such as *CYP1A2* and possibly *ADORA2*, which are associated with caffeine metabolism, sensitivity and response (51).

Over 95% of caffeine is metabolized by the CYP1A2 enzyme, which is encoded by the *CYP1A2* gene (52). The−163A>C (rs762551) single nucleotide polymorphism (SNP) has been shown to alter CYP1A2 enzyme activity (53–55), and has been used to identify individuals as "fast" or "slow" metabolizers of caffeine. Individuals who are considered slow metabolizers, that is with the AC or CC genotype, have an elevated risk of myocardial infarction (56), hypertension and elevated blood pressure (57, 58), and pre-diabetes (59), with increasing caffeinated coffee consumption, whereas those with the AA genotype (fast metabolizers) do not appear to carry these risks.

The largest caffeine and exercise study to date (19), examined the effects of caffeine and *CYP1A2* genotype, on 10-km cycling time trial performance in competitive male athletes after ingestion of caffeine at 0 mg, 2 mg (low dose) or 4 mg (moderate dose) per kg body mass. There was a 3% improvement in cycling time in the moderate dose in all subjects, which is consistent with previous cycling time trial studies using similar doses (46, 60). However, there was a significant caffeine-gene interaction where improvements in performance were seen at both caffeine doses, but only in those with the AA genotype who are "fast metabolizers" of caffeine. In that group, a 6.8% improvement in cycling time was observed at 4 mg/kg, which is >2–4% mean improvement seen in several other cycling time trial studies, using similar doses (46, 60–65). Among those with the CC genotype, 4 mg/kg caffeine impaired performance by 13.7%, and in those with the AC genotype there was no effect of either caffeine dose (19). The findings are consistent with a previous study (20), which observed a caffeine-gene interaction and improved time trial cycling performance with caffeine only in those with the AA genotype.

Some previous endurance-type studies either did not observe any impact of the *CYP1A2* gene on caffeine-exercise studies (66, 67), or reported benefits only in slow metabolizers (45). There are several reasons that may explain discrepancies in study outcomes including smaller sample sizes (<20 subjects) that cause very low numbers and/or no subjects with the CC genotype (45, 67, 68), and shorter distance or different type (power vs. endurance) of performance test (45), compared to those that reported improved endurance after caffeine ingestion in those with the AA genotype of *CYP1A2* (19, 20). The effects of genotype on performance appear to be most prominent during exercise of longer duration or an accumulation of fatigue (aerobic or muscular endurance) (69, 70). Fast metabolizers may quickly metabolize caffeine and achieve the benefits of caffeine metabolites as exercise progresses, or override the short duration of negative impacts (the initial stages of exercise), whereas the adverse effects of restricted blood flow and/or other impacts of adenosine blockage in slow metabolizers are likely to remain for a longer duration (71, 72). Indeed, in a study of basketball performance in elite players, caffeine improved repeated jumps (muscular endurance; an accumulation of fatigue), but only in those with the AA genotype, however, there was no genotype effect in the other two performance components of the basketball simulation (73). Similarly, a cross-over design of 30 resistance-trained men found that caffeine ingestion resulted in a higher number of repetitions in repeated sets of three different exercises, and for total repetitions in all resistance exercises combined, which resulted in a greater volume of work compared to placebo conditions, but only in those with the *CYP1A2* AA genotype (74). Taken together, the weight of the evidence supports the role of *CYP1A2* in modifying the effects of caffeine ingestion on aerobic or muscular endurance-type exercise.

The *ADORA2A* gene is another potential genetic modifier of the effects of caffeine on performance. The adenosine A_{2A} receptor, encoded by the *ADORA2A* gene, has been shown to regulate myocardial oxygen demand and increase coronary circulation by vasodilation (71, 72). The A_{2A} receptor is also expressed in the brain, where it regulates glutamate and dopamine release, with associated effects on insomnia and pain (75, 76). The antagonism of adenosine receptors by caffeine could differ by *ADORA2A* genotype, resulting in altered dopamine signaling (51). Dopamine has been associated with motivation and effort in exercising individuals, and this may be a mechanism by which differences in response to caffeine are manifested (77–79).

One small pilot study has examined the effect of *ADORA2A* genotype (rs5751876) on the ergogenic effects of caffeine under exercise conditions (80). Twelve female subjects underwent a double-blinded, crossover trial comprising two 10-min cycling time trials following caffeine ingestion or placebo. Caffeine benefitted all six subjects with the TT genotype but only one of the six C allele carriers. Further studies are needed to confirm these preliminary findings and include a larger sample to distinguish any effects between the different C allele carriers (i.e., CT vs. CC genotypes).

Sleep is recognized as an essential component of physiological and psychological recovery from, and preparation for, high-intensity training in athletes (81, 82). The *ADORA2A* rs5751876 genotype has also been implicated, by both objective and subjective measures, in various parameters of sleep quality after caffeine ingestion in several studies (83–86). Adenosine promotes sleep by binding to its receptors in the brain, mainly A_1 and A_{2A} receptors, and caffeine reverses these effects by blocking the adenosine receptor, which promotes wakefulness (83). This action, as well as the potency of caffeine to restore performance (cognitive or physical) in ecological situations, such as highway-driving during the night (87), support the notion that the adenosine neuromodulator/receptor system plays a major role in sleep–wake regulation. This action of caffeine may also serve athletes well under conditions of jetlag, and irregular or early training or competition schedules. Psychomotor speed relies on the ability to respond, rapidly and reliably, to randomly occurring stimuli which is a critical component of most sports (88). Genetic variation in *ADORA2A* has been shown to be a relevant determinant of psychomotor vigilance in the rested and sleep-deprived state and modulates individual responses to caffeine after sleep deprivation (85). In support of this notion, individuals who had the TT genotype for *ADORA2A* rs5751876 consistently had faster response times (in seconds) than C allele carriers after ingesting

400 mg caffeine during a sustained vigilant attention task after sleep loss (85).

Consistent with the "adenosine hypothesis" of sleep where the accumulation of adenosine in the brain promotes sleep, caffeine prolongs the time to fall asleep, decreases the deep stages of non-rapid-eye movement (nonREM) sleep, reduces sleep efficiency, and alters the waking and sleep electroencephalogram (EEG) frequencies, which reliably reflect the need for sleep (89–91). Although additional research in this area is warranted, genetic variation appears to contribute to subjective and objective responses to caffeine on sleep. Carriers of the *ADORA2A* (rs5751876) C allele have greater sensitivity toward caffeine-induced sleep disturbance compared to those with the TT genotype (84). Taken together, it appears that individuals with the TT genotype for the rs5751876 SNP in the *ADORA2A* gene may have better performance outcomes, faster response times and less sleep disturbance following caffeine ingestion.

Vitamin A

No studies have examined the role of genetic modifiers of vitamin A status directly on athletic performance, however, there are several important functions of this micronutrient that are associated with optimal health, immunity and performance in athletes.

Vitamin A is a fat-soluble vitamin, which plays a key role in both vision (92) and immunity (93) in its biologically active forms (retinal and retinoic acid). Vitamin A has diverse immune modulatory roles; hence, vitamin A deficiency has been associated with both immune dysfunctions in the gut, and several systemic immune disorders (93). Vitamin A is also a powerful antioxidant, protecting eyes from ocular diseases and helping to maintain vision (92).

High-performance athletes appear to have superior visual abilities based on their capacity to access distinct visual skills, such as contrast sensitivity, dynamic acuity, stereoacuity, and ocular judgment, needed to accomplish interceptive actions (e.g., hand-eye coordination) and resolve fine spatial detail, which is required by many sports (94, 95). In addition, slow visuomotor reaction time (VMRT) has been associated with musculoskeletal injury risk in sporting situations where there are greater challenges to visual stimulus detection and motor response execution (96). These visuomotor skills are key contributors to enhanced sport performance, and accordingly, require exceptional eye health.

Deficiencies of certain micronutrients such as vitamin A decrease immune defense against invading pathogens and can cause the athlete to be more susceptible to infection. Low energy availability (dieting), poor food choices, jetlag, physical and psychological stress, and exposure to pollution and foreign pathogens in air, food and water while traveling can result in a deterioration in immune function and increased susceptibility to illness (97). Athletes following high volume, high intensity training and competition schedules are also known to have more frequent upper respiratory tract infections (URTI) compared to both sedentary and moderately exercising populations (97).

Upon absorption, provitamin A carotenoids are readily converted to vitamin A by the BCMO1 enzyme expressed in enterocytes of the intestinal mucosa (98). β-Carotene is the most abundant provitamin A carotenoid in the diet and the conversion of beta-carotene to retinal or retinoic acid is necessary for vitamin A to exert its biological functions. The rs11645428 variant in the *BCMO1* gene affects circulating plasma carotenoid levels by impacting the conversion of dietary provitamin A carotenoids to active forms of vitamin A in the small intestine (99). Individuals with the GG genotype are inefficient at this conversion, and may be at higher risk for vitamin A deficiency (100). These individuals are considered low responders to dietary β-carotene so consuming enough dietary pre-formed vitamin A (or supplements for vegans), can help to ensure that circulating levels of active vitamin A are adequate to support vision, immunity and normal growth and development.

Anemia-Related Micronutrients: Iron, Folate, and Vitamin B_{12}

There is an abundance of research demonstrating the adverse effects of low iron storage and anemia on athletic performance (23, 101–103). The estimated prevalence of anemias and low levels of iron, folate, and vitamin B_{12} appear to be higher in elite-level athletes than in the general population, and these deficiencies can have significant negative impacts on performance (22, 23, 104–107). The most common symptoms of this disorder are fatigue, weakness and, in extreme cases, shortness of breath or palpitations (103).

The importance of iron to athletes is established through its biological role in supporting the function of proteins and enzymes essential for maintaining physical and cognitive performance (108). Iron is incorporated into hemoglobin and myoglobin, proteins responsible for the transport and storage of oxygen. Iron-deficiency anemia is the most common type of anemia among athletes, who have higher iron requirements due to increased erythropoietic drive through higher intensities and volumes of training. The female athlete is at particular risk of iron deficiency due to menstruation and generally, a lower total energy or food intake compared to males (107, 109). Along with dietary intake, footstrike hemolysis, gastrointestinal bleeding, exercise-induced inflammation, non-steroidal autoinflammatory drug (NSAID) use and environmental factors such as hypoxia (altitude), may influence iron metabolism in athletes of both sexes (23). Macrocytic anemias, which occur when erythrocytes are larger than normal, are generally classified into megaloblastic or nonmegaloblastic anemia. Megaloblastic anemia is caused by deficiency or impaired utilization of vitamin B_{12} and/or folate, whereas non-megaloblastic macrocytic anemia is caused by various diseases, and will not be discussed here (110). Other factors that are associated with anemia risk include genetic variation, which can alter micronutrient metabolism, transport or absorption, and can be used to identify individuals at risk of inadequate levels of vitamin B_{12}, folate and iron stores.

Performance improvements are usually seen with the treatment of anemia (23, 103, 104), which is related to improvements in symptoms such as general feelings of fatigue and weakness, difficulty exercising, and in more severe cases, dyspnea and palpitations (103). Hyperhomocysteinemia, which

can result from low folate and/or vitamin B_{12} intake, may also increase the risk of skeletal muscle malfunction, including muscle weakness and muscle regeneration, and will be discussed further below (111).

Folate

Methylene tetrahydrofolate reductase (MTHFR) is the rate-limiting enzyme in the methyl cycle, and is encoded by the *MTHFR* gene (112). The C677T (rs1801133) polymorphism in the *MTHFR* gene has been associated with low serum and red blood cell folate as well as elevated plasma homocysteine levels, which is an independent risk factor for cardiovascular disease (CVD) (113, 114). Several studies in athletic and non-athletic populations have shown that individuals with the CT or TT genotype are at an increased risk of low circulating folate levels when their diet is low in folate (115–118).

Although there are no studies examining performance outcomes related to *MTHFR* genotypes or dietary folate intake, hyperhomocysteinemia has been shown to be associated with diminished muscle function (111). Several studies conducted in older adults have found a significant association between elevated plasma homocysteine concentrations and declined physical function (119–122), which may be mediated by a reduction in strength (120). Compared to those with the rs1801133 CC genotype, individuals with TT genotype and possibly the CT genotype may be at a greater risk for hyperhomocysteinemia, although this may not be causative for lower physical performance (111, 119, 120). However, soccer players and sedentary individuals with the CC genotype have been shown to have more favorable body composition and performance measures such as aerobic and anaerobic threshold rates, compared to carriers of the T allele (118).

Iron Overload

Genetic variation associated with serum iron levels involves several genes such as *HFE, TMPRSS6, TFR2*, and *TF* (25, 117, 123–128). The *HFE* gene is involved in the regulation of intestinal iron uptake (129), and variations in this gene, which are not very common, have been shown to increase the risk for hemochromatosis or iron overload (124, 130). Excess iron may be toxic to tissues and cells because highly reactive "free" iron reacts with reactive oxygen species (ROS) such as superoxide and hydrogen peroxides, or lipid peroxides to produce free radicals (131). In turn, these free radicals can cause cell and tissue damage (including muscle) and, ultimately, lead to cell death (132). Elevated biomarkers of iron such as ferritin and transferrin are more common in those who are genetically predisposed to iron overload based on the *HFE* gene variant (22, 124). Interestingly, athletes with the rare *HFE* (rs1800562) AA genotype, which is associated with an increased risk for hemochromatosis, may be at a genetic advantage to excel in sport if iron levels are at the high end of the normal range, but not excessive to cause tissue damage. Notably, several studies have found that certain variants of the *HFE* gene that increase risk of iron overload are more common in elite-level athletes compared to the general population, suggesting this may be beneficial for performance (133–135).

Two SNPs in the *HFE* gene (rs1800562 and rs1799945) can be used to predict risk of hereditary hemochromatosis. Based on the combination of variants from these two SNPs, individuals can be categorized as having a high, medium, or low risk for iron overload (124, 128). While genetic risk for iron overload may have a favorable impact on performance, it is necessary for athletes with a medium or high risk to avoid iron supplementation as this could lead to adverse health outcomes (124) and diminished performance.

Low Iron Status

Three main SNPs: *TMPRSS6* (rs4820268), *TFR2* (rs7385804), *TF* (rs3811647) can be used to assess genetic risk for low iron status, primarily due to their involvement in regulating the expression of hepcidin, which is a peptide hormone that controls iron absorption (25, 123, 127). Iron-deficiency anemia impairs performance by reducing oxygen-carrying capacity, but a number of reports indicate that iron deficiency without anemia may affect physiological performance and work capacity as well (21), particularly in women who experience iron deficiency more frequently (22, 23).

There is a fine balance in achieving and maintaining adequate, but not excessive, iron levels for optimal performance. Individuals with the GG genotype in the *TMPRSS6* gene have an increased risk of low transferrin saturation and hemoglobin, compared to those who are carriers of the A allele (25, 26, 123, 136). In the *TF* gene, individuals have a greater risk for low ferritin and elevated transferrin when they possess the AA genotype (25, 123, 136). Variation in the *TFR2* gene can impact hematocrit, mean corpuscular volume, and red blood cell count where individuals with the CC genotype have an increased risk of low serum levels (25). Utilizing algorithms to assess various genotype combinations, these genes can help to determine an individual's overall risk for low iron status, which can lead to iron-deficiency anemia, and can be used to target dietary iron intake. Although iron supplementation is common and frequently prescribed in athletes, many individuals are at risk of taking iron supplements in excess (22, 137, 138). Although iron supplements are commonly "prescribed" by healthcare professionals and nutritionists (139, 140), excess iron stored in skeletal muscle may not only be dangerous to the health of the athlete (124, 141), but also can lead to oxidative stress and the formation of free radicals, and reduced athletic performance (135, 142, 143).

Vitamin B_{12}

Vitamin B_{12} is also associated with RBC formation and aerobic capacity. Megaloblastic anemia results from vitamin B_{12} deficiency and is associated with elevated homocysteine, and results in general feelings of fatigue and weakness. Megaloblastic anemia limits the blood's oxygen carrying capacity, thus reducing its availability to cells (144). Variation in the *FUT2* gene (rs602662) has a significant impact on serum B_{12} levels where individuals with GG or GA genotypes possess the greatest risk for low serum vitamin B_{12} levels, but only when the diet is low

in bioavailable sources of vitamin B_{12} (145). This is consistent with previous genome-wide association studies, which found that individuals with the AA genotype had significantly higher concentrations of serum vitamin B_{12} compared to carriers of the G allele (145).

Vitamin C

Vitamin C is a water-soluble antioxidant that aids in the reduction of exercise-induced free-radical production (146). The production of potentially harmful ROS (147–149) in athletes is greater than in non-athletes due to the massive increases (up to 200-fold at the level of skeletal muscle) in oxygen consumption during strenuous exercise (146, 150). Vitamin C supplementation was once thought to mitigate this risk; however, studies have shown that excess vitamin C supplementation during endurance training can blunt beneficial training-induced physiological adaptations, such as muscle oxidative capacity and mitochondrial biogenesis and may actually diminish performance (148, 149, 151, 152). Dietary consumption of vitamin C, up to 250 mg daily from fruits and vegetables, is likely sufficient to reduce oxidative stress without having a negative effect on performance (151, 153). Additionally, collagen is a key constituent of connective tissue such as tendons and ligaments, and vitamin C is necessary for collagen production. This suggests that vitamin C may play a role in muscle growth and repair (154, 155). Indeed, a recent landmark study examining collagen synthesis in athletes, reported that adding a gelatin and vitamin C supplement to an intermittent exercise protocol improves collagen synthesis and could play a beneficial role in injury prevention and accelerate musculoskeletal, ligament, and/or tendon tissue repair (155).

The relationship between dietary vitamin C and circulating levels of ascorbic acid depend on an individual's *GSTT1* genotype (156). Individuals who do not meet the Recommended Dietary Allowance (RDA) for vitamin C are significantly more likely to be vitamin C deficient (as assessed by serum ascorbic acid levels) than those who meet the RDA, but this effect is much greater in individuals with the *GSTT1* Del/Del genotype than those with the Ins allele (156).

Genetic testing can help to identify athletes who may be at the greatest risk of low circulating vitamin C (ascorbic acid) levels in response to intake. These low circulating ascorbic acid levels may, in turn, diminish performance through an increased risk of high ROS and diminished muscle or connective tissue repair. Although studies have identified associations between circulating ascorbic acid concentrations and vitamin C transporters, $SVCT_1$ and $SVCT_2$, which are encoded by *SLC23A1* and *SLC23A2* (157), there is no evidence that response to vitamin C intake differs by genotype (158). As such, the use of variants in *SLC23A1* and *SLC23A2* to make personalized dietary recommendations is not supported by the studies to date.

Vitamin D

There are no studies that link genetic modifiers of vitamin D status on athletic performance outcomes; however, there are several functions of this vitamin that are associated with bone health, immunity, recovery from training and various performance variables. Genetic determinants of circulating 25-hydroxyvitamin D (25(OH)D) can influence each of these factors thereby influencing performance.

Vitamin D is essential to calcium metabolism, increasing calcium absorption for optimal bone health (1), which is relevant to all athletes, but particularly those participating in sports with a high risk of stress fracture (159–161). Research comparing individuals with sufficient levels to insufficient or deficient levels of 25(OH)D has shown that it helps to prevent injury (159–161), promote larger type II muscle fiber size (24), reduce inflammation (162), reduce risk of acute respiratory illness (159, 160) enhance functional rehabilitation (162), thereby optimizing recovery and acute adaptive responses to intense training through reduced inflammation and increased blood flow (163, 164).

Two genes that have been shown to impact vitamin D status are the *GC* gene and the *CYP2R1* gene (165, 166). Variations in the *GC* and *CYP2R1* genes are associated with a greater risk for low serum 25(OH)D. In one study (165), where 50% of participants took vitamin D supplements, only 22% of the participants had sufficient serum 25(OH)D levels. In the remaining 78% who had insufficient levels, also only about half (47%) took vitamin D supplements. Within this population, vitamin D supplementation only explained 18% of the variation, compared to 30% from genetics, suggesting that genetics may play a greater role than supplementation in determining risk for low 25(OH)D levels (165). Out of the four genotypes analyzed, only *CYP2R1* (rs10741657) and *GC* (rs2282679) were significantly associated with vitamin D status. Specifically, participants with the GG or GA genotype of *CYP2R1* (rs10741657) were nearly four times more likely to have insufficient vitamin D levels. Those with the GG genotype of the *GC* gene (rs2282679) were significantly more likely to have low vitamin D levels compared to those with the TT genotype (165). These results were consistent with findings from previous studies, including the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits (SUNLIGHT), which found significance on a genome-wide basis in 15 cohorts with over 30,000 participants between three genetic variants including *CYP2R1* (rs10741657) and *GC* (rs2282679) on vitamin D status. Not surprisingly, the number of risk variants that the participants possessed was directly related to their risk for vitamin D insufficiency (166). These findings demonstrate that genetic variation may be more impactful than supplementation intakes and behaviors on determining risk for vitamin D insufficiency.

Calcium

Although studies linking calcium intake, genetics and bone fracture has not been conducted in athletes specifically, genetic variation as it relates to risk of calcium deficiency and fracture risk have been studied in a large cohort of individuals, described below (167). Calcium is necessary for growth, maintenance and repair of bone tissue and impacts maintenance of blood calcium levels, regulation of muscle contraction, nerve conduction, and normal blood clotting (168). In order to absorb calcium, adequate vitamin D intake is also necessary. Inadequate dietary calcium and vitamin D increases the risk of low bone mineral density

(BMD) and stress fractures. Low energy intakes, and menstrual dysfunction in female athletes, along with low vitamin D and calcium intakes further increase the risk of stress fractures in both males and females (169–171), and stress fractures are common and serious injuries in athletes (172).

Some individuals do not utilize dietary calcium as efficiently as others and this may depend on variations in the *GC* gene. In one study (167), subjects ($n = 6{,}181$) were genotyped for two SNPs in the *GC* gene, rs7041 and rs4588, and calcium intake was assessed in relation to the participants' risk for bone fracture (167). In the entire sample of participants, only a small increased risk of bone fracture was observed for individuals homozygous for the G allele of *GC* (rs7041) and the C allele of *GC* (rs4588). However, in participants with low dietary calcium intake (<1.09 g/day) and who were homozygous for the G allele of rs7041 and the C allele of rs4588, there was a 42% increased risk of fracture compared to other genotypes. No differences between genotypes were found in participants with high dietary calcium intakes (167). These findings suggest that calcium intake recommendations could be based on *GC* genotype in athletes to help prevent stress fracture.

Choline

Choline was officially recognized as an essential nutrient by the Institute of Medicine (IOM) in 1998 (173). Choline plays a central role in many physiological pathways including neurotransmitter synthesis (acetylcholine), cell-membrane signaling (phospholipids), bile and lipid transport (lipoproteins), and methyl-group metabolism (homocysteine reduction) (174). Human requirements for choline are dependent on gender, age and physical activity level as well as genetics. Choline is produced in the body in small amounts, however, *de novo* synthesis of choline alone is not sufficient to meet human requirements for optimal health (174, 175). The liver and muscles are the major organs for methyl group metabolism, and choline deficiency has been shown to cause both liver and muscle damage (176, 177). Signs of choline deficiency are identified through elevated serum creatine phosphokinase (CPK), a marker of muscle damage (178, 179), and abnormal deposition of fat in the liver, which may result in non-alcoholic fatty liver disease (NAFLD) (176, 180). Reductions in plasma choline associated with strenuous exercise such as triathlons and marathon running have been reported (181, 182). Acetylcholine, a neurotransmitter involved in learning, memory, and attention, depends on adequate choline and a reduction in the release of this neurotransmitter may contribute to the development of fatigue and exercise performance impairment (181–183). Choline supplementation may also improve lipid metabolism, as it has been associated with more favorable body composition (184) and the ability to aid rapid body mass reduction in weight class sports (185).

Common genetic variants in choline (*PEMT gene*) and a folate pathway enzyme *(MTHFD1)* have been shown to impact the metabolic handling of choline and the risk of choline deficiency across differing nutrient intakes (178, 186, 187). The relationship between genetic variants in folate metabolism and choline requirement may arise from the overlapping roles of folate and choline in methionine and phosphatidylcholine (PC) biosynthesis. PC is critical for the structural integrity of cell membranes and cell survival, and methionine is an essential amino acid that plays a critical role in human metabolism and health (187, 188). The *MTHFD1* rs2236225 SNP, which is associated with folate metabolism, has been shown to increase the demands for choline as a methyl-group donor, thereby increasing dietary requirements for this nutrient (188). Individuals that are A allele carriers of the *MTHFD1* gene have been shown to develop signs of choline deficiency and organ (liver and muscle) dysfunction compared to those with the GG genotype (186, 188, 189).

While humans can make choline endogenously *via* the hepatic phosphatidylethanolamine *N*-methyltransferase (*PEMT*) pathway, a SNP in the *PEMT* gene (rs12325817) has been shown to influence the risk of choline deficiency and the partitioning of more dietary choline toward PC biosynthesis at the expense of betaine synthesis (used a methyl donor) (186). Individuals who are C allele carriers of the *PEMT* gene have been shown to develop signs of choline deficiency and organ (liver and muscle) dysfunction compared to those with the GG genotype (178).

Athletes by nature experience muscle damage through high volume and high intensity training (195). A deficient or suboptimal status of choline may place additional stressors on an athlete's ability to recover, repair and adapt to their given training stimulus.

Macronutrients and Body Composition

Several aspects of physique such as body size, shape and composition contribute to the success of an athlete, in most sports. In the athletic population, body composition is often the focus for change, as it can be easily manipulated through diet as both total energy intake and macronutrient composition (192, 196). Variations in macronutrient intake can significantly impact both body fat percentage and lean mass (29, 190, 193, 197–199), as well as performance, where macronutrient manipulation has long been used to partition calories to be used for specific goals across different sports (196).

Although research examining dietary factors and genetics has revealed that manipulation of dietary fat and protein intakes may have greater modifying effects on body composition than carbohydrates, all macronutrients serve a critical purpose. Carbohydrates provide a key fuel for the brain, CNS and working muscles, and the amount and timing of intake impacts sport performance over a large range of intensities (200, 201). Adequate dietary protein is essential for strength and lean body mass accretion, while also playing a relevant role in preserving lean body mass during caloric restriction and immune function (29, 202, 203). Dietary fat provides energy for aerobic activities and is required for the absorption of fat-soluble vitamins (204). Recent research shows that percent energy intake from protein and fat can be targeted to the individual based on genetic variation for optimizing body weight and composition (190, 193, 197–199). Percent energy from carbohydrates should be guided by fuel needs for training and competition while also considering targeted protein and fat intakes based on genetic variation.

Protein

The *FTO* gene is also known as the 'fat mass and obesity-associated gene' since it has been shown to impact weight management and body composition (194, 199, 205, 206). Dietary interventions may mitigate genetic predispositions associated with a higher body mass index (BMI) and body fat percentage, as determined by genetic variation in the *FTO* gene. Specifically, the Preventing Overweight Using Novel Dietary Strategies (POUNDS Lost) multicenter trial found that carrying an A allele of the *FTO* gene (rs1558902–a surrogate marker for rs9939609) and consuming a high protein diet was associated with a significantly lower fat mass at the 2-year follow up period compared to carrying two T alleles. Importantly, participants with the AA genotype (lesser effects in those with AT genotype) who were following the high protein diet protocol had significantly greater losses of total fat mass, total adipose tissue, visceral adipose tissue, lower total percent fat mass and percent trunk fat, compared to those following a lower protein diet protocol (199). Other studies have shown similar results where dietary protein intake was shown to be protective against the effect of the *FTO* risk variants on BMI and waist circumference (194). A randomized controlled trial (RCT) in 195 individuals showed that a hypocaloric diet resulted in greater weight loss in rs9939609 A allele carriers than noncarriers in both higher and lower protein diets, although metabolic improvements improved in all genotypes in the higher protein diets (205). Athletes who possess the AA genotype of the *FTO* gene at rs1558902 would benefit the most in terms of consuming a moderate-to-high protein diet (at least 25% of energy from protein) to optimize body composition. Greater lean mass in athletes has been associated with improved performance in strength and power sports, as well as some endurance events, and a decreased risk for injuries (191, 207). For those athletes who do not possess the response variant (i.e., greater fat loss with higher protein intakes), following a diet with moderate protein intake (∼15–20% energy), to achieve and maintain an ideal body composition is important to note, as excess protein calories may be counterproductive toward this goal. In this instance, dietary goals for optimal performance may be better met by substituting protein energy for other macronutrients such as carbohydrates for fuel, fiber, prebiotics and other micronutrients, or by increasing intakes of essential fats.

Dietary Fat

Dietary fat, an essential component of the human diet, provides energy for aerobic endurance exercise and is necessary for the absorption of the fat-soluble vitamins A, D, E, and K. Independent of total energy intake, the percentage of energy derived from fat in an athlete's diet can impact body composition, based on genetic variation (204). Individuals possessing the TT genotype of *TCF7L2, transcription factor 7 like 2,* at rs7903146 appear to benefit from consuming a lower percent of total energy from fat (20–25% of energy) to optimize body composition (198). Specifically, participants with the TT genotype lost more fat mass when they were consuming a low-fat diet, compared to a high-fat diet (40–45% of energy) (198). Moreover, individuals with the CC genotype in rs7903146 who consumed lower-fat diets actually lost significantly more lean mass, suggesting that these individuals should avoid low-fat nutrition interventions (197) in order to optimize body composition for athletic performance (191, 207). Body composition can, therefore, be optimized by targeting fat intake based on genetic variation in the *TCF7L2* gene.

Monounsaturated Fat

Recommendations for fat intake can be further targeted to the different types of fats comprising total dietary fat. Athletes with the GG or GC genotype of the *PPARγ2* gene at rs1801282 would benefit from a weight loss intervention that specifically targets body fat, while preserving lean body mass. Such individuals have been shown to demonstrate an enhanced weight loss response when consuming > 56% of total fat from monounsaturated fatty acids (MUFAs) compared to those with the GG or GC genotype who consume < 56% of total fat from MUFAs. These results have not been found in those with the CC genotype of *PPARγ2* at rs1801282 (208).

MUFAs can be targeted in athletes who are aiming to decrease their body fat. It is well-known that a lower body fat percentage is associated with enhanced performance in most sports (191, 207), however, sport clinicians must be cautious about nutrition recommendations aimed at reducing body fat. Striving for very low levels of body fat is highly correlated with the Relative Energy Deficiency in Sport (RED-S) syndrome in both females and males, which refers to 'impaired physiological functioning caused by relative energy deficiency and includes impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health (209).

Saturated Fat and Polyunsaturated Fat

A nested case-control study found that the ratio of dietary saturated fatty acids (SFA) to polyunsaturated fatty acids (PUFA) influenced the risk of obesity associated with the TA and AA variants of the *FTO* gene at rs9939609 (210). Specifically, participants possessing the A allele had a significantly higher BMI and waist circumference (WC) compared to TT homozygotes, but only when intakes of SFA were high and PUFAs were low. When participants with the A allele consumed < ∼15% of energy from SFA and had a higher dietary PUFA:SFA ratio, there were no significant differences in WC and BMI between this group and participants with the TT genotype of rs9939609 (210). These findings have implications for nutrition counseling impacting body composition (abdominal fat specifically) and BMI. Athletes with the TA or AA genotype may have a greater risk for accumulating excessive abdominal fat. An athlete can mitigate this risk by aiming to consume <10% of energy from SFA (to also account for heart health) and > 4% of energy from PUFAs, resulting in a PUFA:SFA ratio of at least 0.4 (210).

SUMMARY

This paper provided an overview of the current science linking genetic variation to nutritional or supplemental needs with a focus on sport performance. One of the ultimate goals in the field

of personalized sport nutrition is the design of tailored nutritional recommendations to improve direct and indirect factors that influence athletic performance. More specifically, personalized nutrition pursuits aim to develop more comprehensive and dynamic nutritional and supplement recommendations based on shifting, interacting parameters in an athlete's internal and external (sport) environment throughout their athletic career and beyond.

Currently, there are few gene-diet interaction studies that have directly measured performance outcomes and been conducted in competitive athletes, so this should be a focus of future research. However, it has been established that serum levels and/or dietary intakes of several nutrients and food bioactives can impact overall health, body composition and in turn result in modest to sizable modifying effects in athletic performance. The strongest evidence to date appears to be for caffeine on endurance performance with several trials demonstrating the modifying effects of genetic variants with sports performance outcomes. Genetic testing for personalized nutrition may, therefore, be an additional tool that can be implemented into the practice of sport clinicians, nutritionists and coaches to guide nutritional counseling and meal planning with the aim of optimizing athletic performance.

AUTHOR CONTRIBUTIONS

NG and AE-S conceived of the original idea for the review and NG wrote the first draft. All authors contributed to the writing and editing of the manuscript. AE-S secured funding.

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REVIEW
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A Statistical Framework to Interpret Individual Response to Intervention: Paving the Way for Personalized Nutrition and Exercise Prescription

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The concept of personalized nutrition and exercise prescription represents a topical and exciting progression for the discipline given the large inter-individual variability that exists in response to virtually all performance and health related interventions. Appropriate interpretation of intervention-based data from an individual or group of individuals requires practitioners and researchers to consider a range of concepts including the confounding influence of measurement error and biological variability. In addition, the means to quantify likely statistical and practical improvements are facilitated by concepts such as confidence intervals (CIs) and smallest worthwhile change (SWC). The purpose of this review is to provide accessible and applicable recommendations for practitioners and researchers that interpret, and report personalized data. To achieve this, the review is structured in three sections that progressively develop a statistical framework. Section 1 explores fundamental concepts related to measurement error and describes how typical error and CIs can be used to express uncertainty in baseline measurements. Section 2 builds upon these concepts and demonstrates how CIs can be combined with the concept of SWC to assess whether meaningful improvements occur post-intervention. Finally, section 3 introduces the concept of biological variability and discusses the subsequent challenges in identifying individual response and non-response to an intervention. Worked numerical examples and interactive Supplementary Material are incorporated to solidify concepts and assist with implementation in practice.

Keywords: measurement error, biological variability, individual response, typical error, meaningful change, responders

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INTRODUCTION

It is widely recognized that traditional group intervention-based studies that focus on mean response are limited in the context of personalized sports nutrition, and that across populations, large inter-individual variability exists in response to health and performance related interventions. This variation occurs due to a myriad of factors, including an individual's genotype, phenotype, training status, and nutritional intake (1, 2). Accordingly, an increasing number of investigations are attempting to interpret individual data and classify participants as responders or non-responders to nutrition or exercise based interventions (3–11). In order to accurately interpret

individual data collected from group-based interventions it is essential that researchers and practitioners consider a range of concepts including the confounding influence of *measurement error* and *biological variability*. In addition, the ability to interpret practical and statistical significance are enhanced by concepts such as *smallest worthwhile change* (SWC) and *confidence intervals* (CIs). The aim of this review is to describe a statistical framework that can be used by researchers and practitioners in the fields of applied sports nutrition and exercise physiology. The review is structured into three sections that build upon each other and develop into a coherent statistical framework. The initial section introduces concepts from classical test theory (12), namely measurement error, and describes how the calculation of typical error and the application of CIs can be used to express uncertainty in baseline values. Section two of the review builds upon the previous section and demonstrates how CIs can be combined with the concept of SWC to assess whether meaningful changes have occurred following an intervention. The final section of the review discusses the concepts of individual response and non-response and describes how the statistical framework developed can be used to estimate the proportion of response in a group-based intervention.

Key terms that will be used throughout the review have been defined in **Table 1** and are italicized on first use. To facilitate understanding, worked examples are included throughout the review, from a hypothetical randomized controlled study ($n = 20$) investigating the influence of 12 weeks of beta-alanine supplementation on: (1) body composition [assessed by sum of 7 skinfolds]; (2) muscle carnosine content [assessed by high-performance liquid chromatography; HPLC], and (3) high-intensity cycling capacity [assessed by the $CCT_{110\%}$, a time-to-exhaustion test]. The study design is illustrated in **Figure 1**. Mock data from the study along with all worked examples are included in the accompanying supplementary digital file (SF). Automated spreadsheets are also included for readers to incorporate their own data sets and follow the procedures described within this review.

1. ESTABLISHING PLAUSIBLE BASELINE VALUES (TRUE SCORE)

Practitioners and researchers routinely select and evaluate interventions depending on baseline information collected from an individual. Therefore, it is essential to consider the accuracy of baseline information and account for error in any decision-making process. An individual's *true score* can be viewed as their current stable level in the test of interest. In practice, we can never know an individual's true score as all measurement incorporates error and therefore, a single measurement from a test is simply referred to as an *observed score*. In classical test theory, it is assumed that if it were possible to conduct a large number of tests on the same individual then the values observed would follow a normal (Gaussian) distribution, with mean equal to the true score and standard deviation (σ) describing variability around this mean (12). In mathematical notation, we state that the observed score (O_s) comprises a hypothetical true score (T_s) and measurement error (ϵ), such that $O_s = T_s + \epsilon$ (13). This perspective has clear implications when using baseline measurements to select interventions as an individual's true score always remains unknown. For tests that frequently produce large measurement errors, there is greater likelihood that observed scores will differ substantially from the true score, such that conclusions drawn, and interventions adopted may be unnecessary, ineffective, or indeed inappropriate.

Measurement error associated with any test comprises two primary sources, namely *instrumentation noise,* and *biological noise*. Here, we define instrumentation noise as error caused solely by the measurement apparatus. For example, offsets in calibration or variation in saddle position may cause observed performances in a cycling-based test, such as the $CCT_{110\%}$ to differ from the individuals true score (14). In contrast, we define biological noise as error in observed scores caused by biological processes, including, but not limited to, phenomena such as circadian rhythm, nutritional intake, sleep and motivation (1). When selecting and administering tests, every effort should

TABLE 1 | Definitions of key terms.

Term	Definition
True score	A hypothetical value representing the score on a test that would be achieved if there were no measurement error.
Measurement error	Processes that causes an observed score on a test to be different from the true score. Measurement error comprises instrumentation and/or biological noise.
Observed score	The recorded value from a test, which comprises the true score, along with measurement error.
Instrumentation noise	Measurement error caused solely by the measurement apparatus, while true score remains unchanged.
Biological noise	Measurement error caused by biological processes (such as circadian rhythm, nutritional intake, sleep or motivation), while true score remains unchanged.
Typical error	The standard deviation of observed scores in repeated tests where true score remains unchanged.
Confidence interval	An interval that provides a range of plausible values for quantities that must be estimated (for example, true score) given the observed data.
Biological variability	Non-intervention related processes that cause true scores to change.
Smallest worthwhile change	A reference value selected by a practitioner or researcher to indicate a value beyond which a change in true score is likely to be meaningful in practice.
Response	Occurs when change in true score directly attributable to an intervention exceeds the smallest worthwhile change.

be made to minimize the magnitude of measurement error. This can be achieved through adherence to standardized set-up, calibration and testing protocols, along with standardization of external factors likely to impact test scores through the introduction of additional biological noise (e.g., time of testing, nutritional intake and activity performed prior to testing). It is important to acknowledge, however, that these processes can only serve to reduce, but never to eradicate, measurement error.

KEY POINTS:

- *Due to the presence of measurement error, an individual's true score representing their current ability in a test always remains unknown and can only be estimated.*
- *Observed scores comprise the hypothetical true score and measurement error due to instrumentation and biological noise.*

1.1. Calculating the Typical Error of a Test

As all observed measurements include error, it is important to estimate the potential magnitude of this error and thereby quantify uncertainty in any single measurement. Based on the assumption that observed scores follow a normal distribution centered on the true score, ∼68% of observed scores lie in the interval $T_s \pm \sigma$ and ∼95% of observed scores lie in the interval $T_s \pm 2\sigma$ (**Figure 2**). Therefore, the key to quantifying likely measurement error and ultimately providing ranges for true scores consistent with the data, requires estimation of the standard deviation (σ) for repeated tests. In applied physiology literature, this standard deviation is commonly referred to as the *typical error* (15) (TE), and from this point forward we will use the notation TE in all formulae instead of σ.

Two primary methods are available to estimate the TE of a test, including: (1) multiple repeated tests performed by a single individual; or (2) a single test-retest performed by a group of individuals. Using the first approach, the TE is estimated by calculating the standard deviation of observed scores obtained from a single individual performing multiple tests within a time-frame whereby the true score remains theoretically stable. Suitable time-frames will depend on the specific characteristics of a given test. For example, true score in the $CCT_{110\%}$ is largely dependent on the capacity of the cardiovascular and muscular systems, neither of which are likely to undergo substantial physiological changes in the absence of intervention within short time-frames. The true score for $CCT_{110\%}$ performance should therefore remain stable across days or even weeks, although biological noise in particular (e.g., motivational factors), may cause observed scores to fluctuate within this time-frame (16). The accuracy of the TE estimate based on repeated tests conducted with an individual will generally increase with the number of repeated tests but may require more than 10–20 tests to obtain suitable accuracy. This requirement presents logistical difficulties in relation to resources required to repeatedly administer many tests with any one individual. In addition, even if these logistical difficulties could be overcome, the testing process itself may lead to a change in the individual's true score, and as a result, the estimate of the TE will become inflated (15). Continuing the $CCT_{110\%}$ example, repeated performance of a high-intensity activity to exhaustion is likely to create a strong stimulus for adaptation (17), thereby causing true score to change and estimates of variation to subsequently increase. For these reasons, single individual approaches to estimate TE are rarely used in the exercise sciences.

Based upon the aforementioned limitations, the most popular method to estimate the TE of a test relies on multiple individuals each performing a single test-retest assessment (15). This approach is well suited to those working with sport teams or groups taken from a relatively homogenous population. Group based calculations for estimating TE generally rely on the assumption that, whilst true scores may vary between individuals, TE is consistent across the population being assessed. Based on this assumption, if test-retest values are obtained from a group of individuals over a period where true scores are expected to remain constant, TE can be estimated from the difference scores obtained. Difference scores are calculated for each individual in the group test-retest by simply subtracting the observed scores in test 1 from the observed scores in test 2 (i.e., observed[test2]—observed[test1]). These difference scores are described by a normal distribution with mean 0 and standard deviation equal to $\sqrt{2}$TE. The standard deviation is equal to $\sqrt{2}$TE as the variance (which is equal to the square of the standard deviation) of the difference scores is equal to the variation in test 1 plus the variation in test 2 (i.e., $TE^2 + TE^2 = 2TE^2$). Therefore, to obtain the TE estimate with a group test-retest design, we first calculate the difference score for each individual, calculate the standard deviation of the differences scores, then divide this value by $\sqrt{2}$. Formulae and worked examples of this TE calculation are included in the Supplementary File (SF-S2) using test-retest data from sum of skinfolds and muscle carnosine biopsies conducted 48–72 h apart (i.e., a time frame where true score is unlikely to change). For the muscle carnosine data, the standard deviation of the difference scores from the repeated tests was calculated to be 0.74 $mmol \cdot kg^{-1}dm$; hence the estimate of TE is $0.74/\sqrt{2} = 0.52$ $mmol \cdot kg^{-1}dm$. It is important to note that this calculation represents an estimate of TE and is unlikely to exactly match the real value. Therefore, we use the notation $\widehat{TE}$ from this point forward in calculations where we refer to an estimate of TE.

KEY POINTS:

- *Typical error represents the variation in observed scores caused by measurement error when an individual performs repeated tests.*
- *An **estimate** of typical error can be obtained by calculating the standard deviation of repeated tests performed by a single individual, or more commonly using test-retest data from a group over time periods where true scores are not expected to change.*

1.1.1. True Score Confidence Intervals

Once an observed score and TE estimate have been obtained, a *confidence interval* (CI) for the true score can be created. CIs are used to quantify uncertainty in estimates that cannot be directly measured (18). Therefore, calculation of a CI for a true score provides a range of plausible values given the observed data. We have highlighted that conceptually, true score is equal to the mean of a large number of non-interacting repeated tests, and that observed scores follow a normal distribution around this mean. Therefore, measurement error in a single observed score is just as likely to be positive as it is negative. Therefore, true

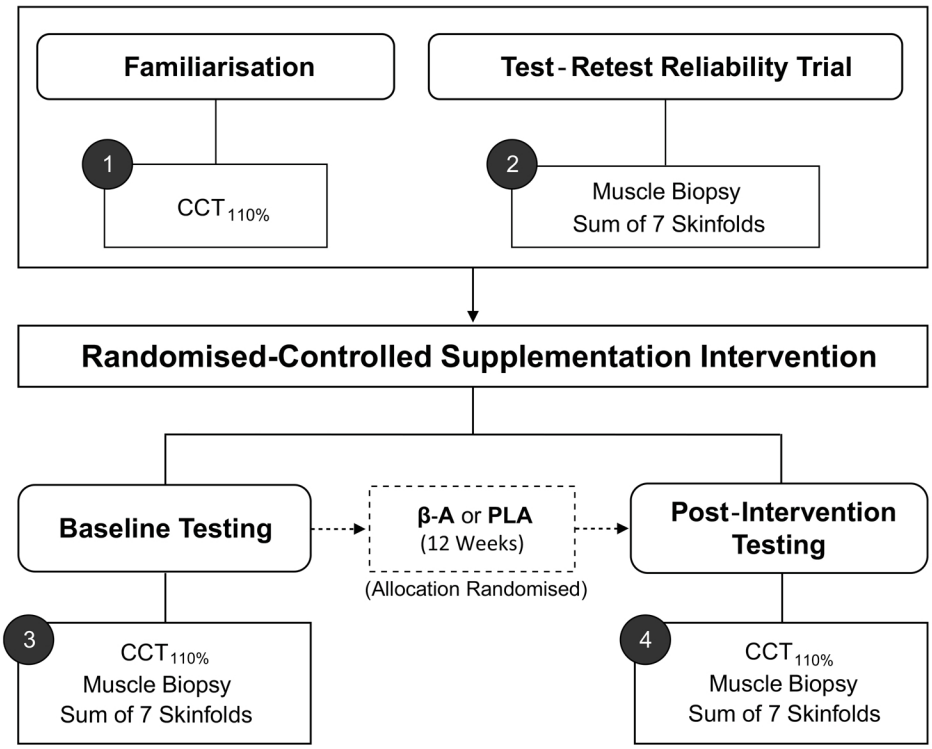

FIGURE 1 | Schematic of hypothetical study design. $CCT_{110\%}$, High-intensity cycling capacity test; B-A, Beta-alanine supplementation; PLA, Placebo.

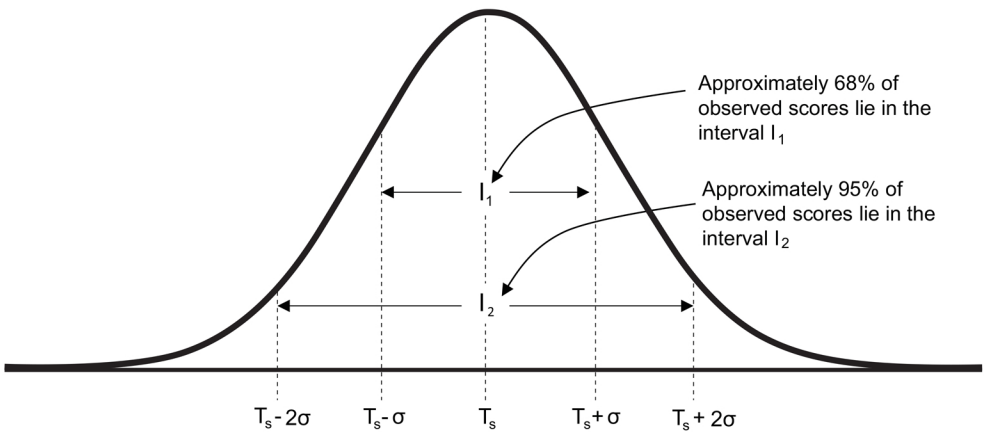

FIGURE 2 | Graphical representation of the normal distribution of observed scores centered on true score. Ts, true score; σ standard deviation of repeated observed scores [also referred to as typical error (TE)].

score CIs are created by adding and subtracting a multiple of the estimated TE to the observed score, with larger multiples creating wider intervals (**Figure 2**). CIs are interpreted as a property of a procedure and when used repeatedly, the percentage of intervals calculated that include the true value being estimated will match the % CI used (19). In other words, if a practitioner routinely follows the procedure of estimating TE and calculating, say, 95% CIs for true scores, then through maintaining a compilation of these values over their career the percentage of intervals that contain the true score will be ∼95%. A key point here is that a CI based on a single dataset should not be interpreted probabilistically (19), as it is possible to obtain a very high, or very low estimate of TE by chance, such that true score CIs calculated will be inappropriate.

1.1.2. Calculating True Score Confidence Intervals of Different Widths

The measurement assumptions outlined in the previous section enable practitioners to calculate various CI widths by multiplying their TE estimate by values that are based on the normal

distribution. In the first row of **Table 2**, we provide the values required to obtain a range of standard CI widths. Returning to our muscle carnosine example where we obtained a TE estimate of 0.52 mmol·kg^{-1}dm, an approximate 95% true score CI for an individual with an observed score of 11.3 would equal 11.3 $\pm$ (1.96 $\times$ 0.52) = (10.3 − 12.3) mmol·kg^{-1}dm. It is important to note that the values provided in the first row of **Table 2** provide only approximate CIs as the TE value describing variation of repeated performances is unknown and only the estimate $\widehat{\text{TE}}$ is available. The accuracy of this estimate depends primarily on the number of individuals (or number of repeated trials) used in the test-retest calculation. CIs based on a TE estimate from smaller samples sizes (e.g., 6–12 participants) will in general, be less accurate than those based on larger sample sizes (e.g., >30). To account for this additional uncertainty, the value used to multiply $\widehat{\text{TE}}$ and obtain a given CI should be adjusted and a larger multiple used. In the subsequent rows of **Table 2**, we present adjusted values that provide more accurate CIs over a range of test-retest sample sizes. Values presented in **Table 2** clearly illustrate that for CI widths close to 50%, the sample size used to estimate TE has minimal effect on the multiple required. However, for wider CIs such as a 95% CI, adjustment for smaller test-retest sample sizes can result in more notable differences. In our previous example (n = 20), the individuals 95% true score CI for muscle carnosine was 10.3 − 12.3 mmol·kg^{-1}DM. In contrast, if $\widehat{\text{TE}}$ was obtained from test-retest with only 5 individuals, the adjusted 95% CI would equal 11.3 $\pm$ (2.78 $\times$ 0.52) = 9.9 − 12.7 mmol·kg^{-1}dm. To identify the number of individuals required for a test-retest, the values presented in **Table 2** can provide insight. Practitioners can make an initial estimate of TE and create for example, 95% true score CIs adjusting for n = 5, 10, 20, 30, and 50. Interpreting the practical relevance of the different CI widths generated can then be used to inform sample size used. For readers that require more detail on the adjustment approach a full explanation of how to obtain the values for any sample size and CI width is presented in Appendix 1. We also present in the Supplementary File an interactive calculator to calculate unadjusted and adjusted CIs of different widths, that can be combined with the mock data set (SF-S4) or a reader's own data set (SF-S5).

KEY POINTS:

- *Confidence intervals can be used to present plausible values of an estimate given the observed data. Repeated application of estimating typical error and associated confidence intervals will result in a match between the percentage of intervals containing the true value and the percentage interval adopted.*
- *True score confidence intervals can be calculated using the observed score and a multiple of the estimated typical error. The multiple selected depends on the desired width of the confidence interval and the number of individuals (or number of repeated tests) used to estimate the typical error.*

1.2. Literature Based Confidence Intervals

In circumstances where it is not feasible to perform repeated measurements on a single individual or group, practitioners can create CIs for true scores using reliability data published in the literature. To obtain accurate CIs it is recommended that practitioners source reliability data collected using the same test protocols employed with their own clients, and that the populations match as close as possible. TE estimates are commonly reported in reliability studies and practitioners can directly use these published values to calculate CIs using the methods described in Section 1.1.1. It is also common for researchers to report other reliability statistics that can be transformed into a TE estimate. One commonly reported reliability statistic that can easily be transformed is the coefficient of variation (CV). The coefficient of variation is a percentage that expresses the size of the TE relative to the mean [$CV\% = (TE/mean) \times 100$; (20)]. Therefore, a true score CI can be obtained using published CV values by first identifying the TE estimate from $\widehat{\text{TE}} = (CV \times O_s)/100$, then applying the procedures outlined in Section 1.1.1. In our hypothetical study, duplicate measurements were not available for the $\text{CCT}_{110\%}$, and therefore we describe here (and in Supplementary File: SF-S6) how to estimate a TE and true score CI based on previously published data. Saunders et al. (21) reported that the CV for total work done in the $\text{CCT}_{110\%}$ was 4.94% (21). Therefore, for an individual with an observed score of 43.0 kJ, we calculate an estimated TE of (4.94 $\times$ 43.0)/100 = 2.1 kJ. Using the values in **Table 2**, we can calculate a range of true score CIs. If we select, say, an unadjusted 75% CI then we would obtain 43.0 $\pm$ (1.15 $\times$ 2.1) = 40.6 − 45.4 kJ. An overall summary of the process for estimating TE and calculating true score CIs is presented in **Figure 3**.

SUMMARY OF KEY POINTS FROM SECTION 1.2:

- *Confidence Intervals can be calculated from literature using published TE values or other reliability statistics (e.g., coefficient of variation (CV)).*

2. ASSESSING WHETHER MEANINGFUL CHANGES HAVE OCCURRED POST-INTERVENTION

As described in the previous section, an individual's true score cannot be known due to the existence of measurement error and this uncertainty must be accounted for when interpreting pre- to post-intervention change. This requirement is particularly relevant in sports nutrition based interventions where improvements are often small in magnitude whilst many performance based outcome measures may be prone to relatively large measurement errors. For example, Jeukendrup et al. (22) showed that a time-to-exhaustion test at 75% of previously determined maximal power output had a CV of 26.6%, which is far in excess of the 5-15% changes in exercise capacity shown with beta-alanine supplementation (23–26). In Section 1.1.2, we used CIs to express our level of uncertainty in baseline test scores. Similarly, CIs can be used to express the level of uncertainty in the change in test scores due to an intervention (*true score change*). Many of the tools and calculations that were introduced in the previous section on baseline scores are also relevant when considering appropriate methods to quantify and interpret change across an intervention. In the following sections we describe minor alterations required to calculate CIs for true score change in comparison to baseline true score. We also

TABLE 2 | Typical error multiples required to calculate confidence intervals of different widths (non-adjusted and adjusted for sample size).

Confidence interval width	50%	60%	70%	75%	80%	85%	90%	95%	99%
TE multiple non-adjusted	0.67	0.84	1.04	1.15	1.28	1.44	1.64	1.96	2.58
TE multiple adjusted ($n = 50$)	0.68	0.85	1.05	1.16	1.30	1.46	1.68	2.01	2.68
TE multiple adjusted ($n = 30$)	0.68	0.85	1.06	1.17	1.31	1.48	1.70	2.05	2.76
TE multiple adjusted ($n = 20$)	0.69	0.86	1.07	1.19	1.33	1.50	1.73	2.10	2.86
TE multiple adjusted ($n = 10$)	0.70	0.88	1.10	1.23	1.38	1.57	1.83	2.26	3.25
TE multiple adjusted ($n = 5$)	0.74	0.94	1.19	1.34	1.53	1.78	2.13	2.78	4.60

introduce the concept of SWC to better assess the effectiveness of an intervention.

2.1. Confidence Intervals for True Score Change

If we assume that measurement error of a test is not only consistent across individuals in a group, but also consistent for individuals across an intervention, then observed scores will display the same variation around the true pre-intervention score and the true post-intervention score. It follows that observed change scores ($OS_{post} - OS_{pre}$) are described by a normal distribution with mean equal to the true score change and standard deviation (i.e., standard deviation of the change scores) equal to $\sqrt{2}$TE. Note, this is the same result discussed in section 1 for the test-retest situation, except here we expect true scores to change due to the intervention. Therefore, to estimate this standard deviation we simply take our previous TE estimate (obtained from repeated tests on a single individual, test-retest for a group, or from published literature) and multiply by $\sqrt{2}$. CIs are then obtained using the procedures outlined in section 1.1.2, except here we apply our estimate around the observed difference across the intervention. For example, in our hypothetical data set, the TE for the muscle carnosine content analysis was 0.52 mmol·kg^{-1}DM, with participant 8 (from the beta-alanine group) displaying an observed change score (difference pre-post) of 4.37 mmol·kg^{-1}DM. For this example, we will calculate an unadjusted 50% true score change CI using the appropriate multiplier presented in **Table 2**. The required calculation is therefore $(OS_{post} - OS_{pre}) \pm \left(0.67 \times \sqrt{2}\widehat{\text{TE}}\right) = 4.37 \pm \left(0.67 \times \sqrt{2} \times 0.52\right) = 3.9 - 4.9$ mmol·kg^{-1}DM. Interactive true score change CI calculators are provided in the Supplementary File for the study mock data (SF-S7) and the readers own data (SF-S8).

SUMMARY OF KEY POINTS FROM SECTION 2:

- *True score change occurs whenever the underlying stable characteristic measured by a test changes.*
- *The estimated typical error can be used to create confidence intervals for true score change pre- to post-intervention.*

2.2. Criteria for Assessing Meaningful Changes and the Smallest Worthwhile Change

In the previous section we described procedures to calculate true score change CIs that provide a range of plausible values given the data observed. In practice, it is recommended that interventions are classified as successful or not for each individual based on whether CIs for true score change lie within a pre-defined region (27). If for example, a practitioner deems that *any* true score change in the desired direction is to be regarded as meaningful, then an intervention for an individual would be classified as successful if both ends of the true score change CI lie to the desired side of the zero line (see **Figures 4A,B**). It has also been suggested that CIs for true score change be calculated based on the observed change plus/minus the estimated TE ($OS_{post} - OS_{pre} \pm \widehat{\text{TE}}$; (27)). This simple calculation provides a close approximation for a 50% true score change CI [*1]. Additionally, with the assumption that observed score change provides a non-biased estimate, we should expect on 50% of occasions for the true score change to lie within the calculated interval, on 25% occasions the true score change to lie below the interval, and on 25% of occasions the true score change to lie above the interval. As a result, if interventions are deemed a success only if observed score change $\pm$ $\widehat{\text{TE}}$ lie to the desired side of the 0 line, then the proportion of times an intervention will correctly be identified as a success will be greater than 75% in the long-run. The accuracy of this statement is illustrated in **Figure 4** using the most conservative successful case, where the approximate 50% CI bound lies on the zero line. Detailed calculations of this process are presented in the Supplementary File (SF-S8), however, we briefly provide an example here. In our hypothetical data set, participant 4 recorded an observed decrease of 7.1 mm in the sum of 7 skinfolds pre- to post-intervention. Additionally, the TE estimate obtained for sum of seven skinfolds from the hypothetical data was 1.35 mm. Therefore, we calculate the approximate 50% true score change CI with $-7.1 \pm 1.35 = (-8.45$ to $-5.75)$ mm. As both the upper and lower bound of the interval are negative (which is the desired direction indicating a reduction in body fat), we conclude that the intervention was successful for this participant.

Thus far in this section, we have focused on scenarios where any true score change greater than 0 in the desired direction is considered meaningful. In many research settings, this approach will be appropriate, given that researchers are likely to deal with experimental scenarios and unknown outcomes. In contrast, in other situations, researchers and practitioners may implement interventions whereby relatively large improvements

[1]The calculation used to obtain a true score change is $\left(OS_{post} - OS_{pre}\right) \pm M\sqrt{2}$TE, where M is the multiple used to set the CI width. With $\left(OS_{post} - OS_{pre}\right) \pm$ TE we have set M to $\left(\frac{1}{\sqrt{2}}\right) = 0.71$, and if we check the unadjusted multiples provided in **Table 2**, this approximately equates to a 50% CI.

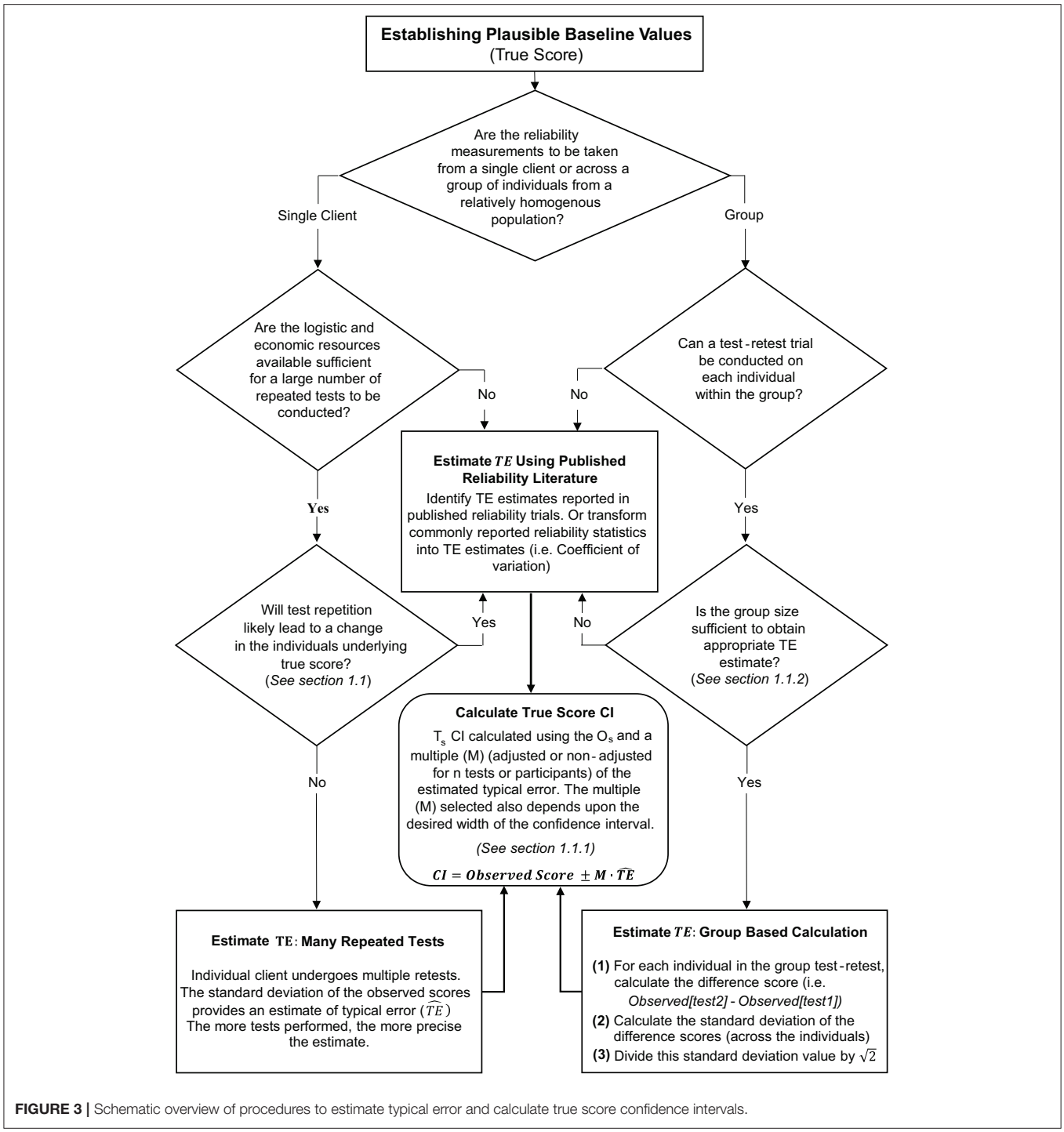

FIGURE 3 | Schematic overview of procedures to estimate typical error and calculate true score confidence intervals.

are expected, such that more substantive changes are required in order to classify an intervention as a success. Take for example our hypothetical intervention, which aims to increase muscle carnosine content through beta-alanine supplementation. Previous investigations indicate that 4 weeks of supplementation can increase muscle carnosine content by 40–60% (28) and more recently, maximal increases ranging from 60 to 200% have been reported for participants supplementing for 24 weeks (26). Increased intramuscular carnosine content causes a subsequent increase in intramuscular buffering capacity, which may counteract high-intensity induced acidosis and thus fatigue (29). Given this context, establishing that a participant experienced a true score increase in intramuscular carnosine just beyond zero would be considered practically meaningless, given

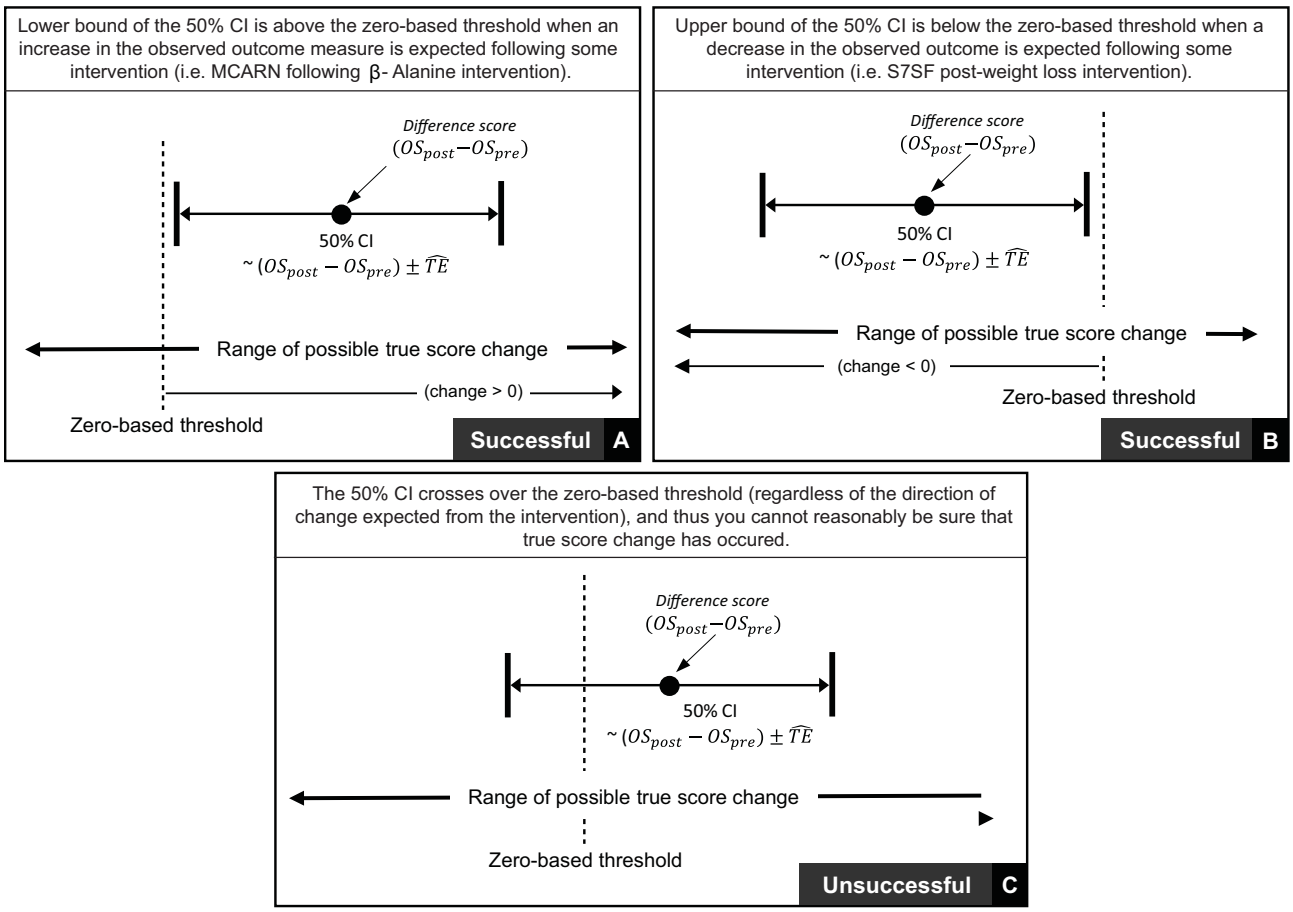

FIGURE 4 | Interpretation of true score change confidence intervals using zero-based thresholds.

the negligible influence on buffering capacity and subsequent high-intensity exercise performance. Instead, researchers and practitioners may choose to identify threshold values beyond zero that represent the smallest change required to be practically relevant. This threshold value is generally referred to as the SWC and is often selected subjectively by practitioners based on what they believe is practically relevant or from their experience working with a particular client base using similar interventions. Alternatively, calculations of effect size (e.g., Cohen's D) can be used to objectively determine the SWC, with a value of 0.2 times the baseline between-individual standard deviation often considered to be appropriate (27). Approaches to calculate SWC are discussed in detail elsewhere (30–32). Once a SWC value has been selected, the general approach to determining meaningful change post intervention remains the same and for each participant an intervention is classified as a success if the true score change CI lies beyond the selected SWC (**Figure 5A**, with non-success illustrated **Figure 5B**).

To effectively implement these procedures, tests that comprise appropriate measurement error relative to the SWC are required. It is recommended when implementing this approach that an *a priori* determination of the SWC deemed practically relevant is made. In most cases, it would be expected that the majority of individuals that engage in an intervention should exceed the SWC, therefore, the threshold set should be below the likely projected change for most individuals. An appropriate test with regards to measurement error would then be one where the TE is no larger than the gap between these two values (namely expected change and SWC). As an example from the hypothetical study considered here, a practitioner may decide to set a SWC threshold for the $CCT_{110\%}$ by multiplying the baseline between-participant standard deviation by 0.2 (i.e., a 'small' effect), providing a value of 1.6 KJ. From previous research, 4 weeks of beta-alanine supplementation has been shown to improve $CCT_{110\%}$ performance by ∼10–15% (23–25). If we consider participant 1 from the supplementation group with a baseline value of 42.8 kJ and expect a realistic 10% improvement, then we project a value of 47.1 kJ by the end of the intervention equalling an improvement of ∼4.3 kJ. Given a TE value of 2.2 kJ and implementing the recommended process, in order to judge the intervention successful, participant 1 would require an observed score improvement of at least $1.6 + 2.2 = 3.8$ kJ (which exceeds the *a priori* set SWC but is also less than what we expect in general). In contrast, if the estimated TE was considerably higher (e.g., due to lack of control for instrumentation or biological noise), say 5 kJ, then the same participant would require an

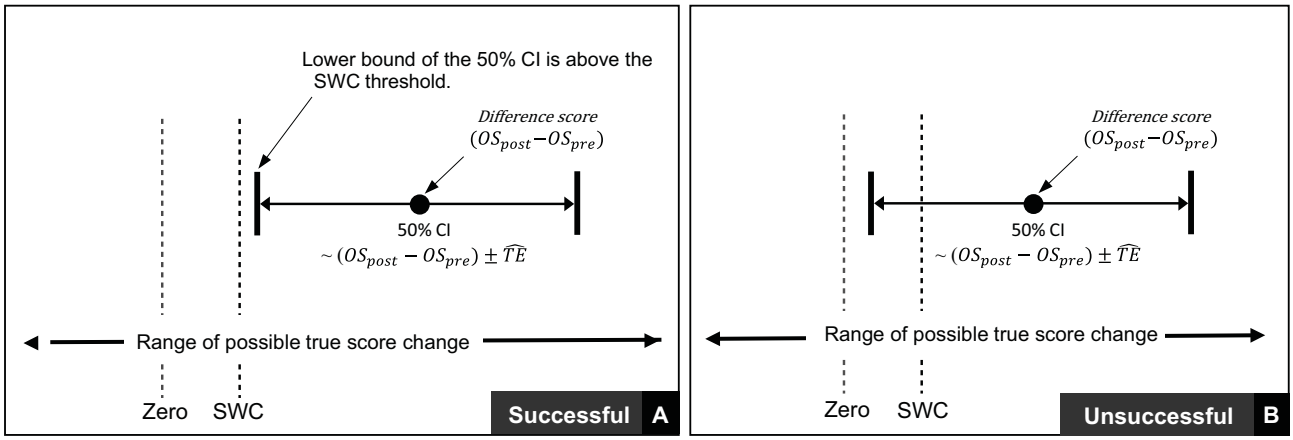

FIGURE 5 | Interpretation of true score change confidence intervals using smallest worthwhile change.

observed score improvement of at least $1.6 + 5 = 6.8$ kJ, which is a larger change than the literature indicates would typically be expected. This approach may therefore frequently lead to interventions being deemed not-successful when in fact the large TE may have masked any detectable improvement. In situations such as this, consideration of factors which may reduce the TE of the test are advised (e.g., enhanced standardization of procedures, repeat familiarisations), however if this is not possible, then this particular test may lack the sensitivity required to detect meaningful changes, and an alternative one may be required. Worked examples of this entire process with mock data are presented in the Supplementary File (SF-S9) along with an interactive calculator that can be used to identify successful interventions with readers own data (SF-S10).

KEY POINTS:

- *When assessing the effectiveness of an intervention, it is recommended that practitioners identify a smallest worthwhile change (which in some cases may be zero).*
- *Practitioners may choose to judge an intervention successful for an individual if the observed score change (post-pre) ± typical error lie beyond the smallest worthwhile change.*
- *Practitioners must ensure that tests include typical error values that are not too large such that individuals require unrealistic improvements for confidence interval bounds to lie beyond smallest worthwhile change values.*

3. RESPONSE TO INTERVENTION AND THE ROLE OF BIOLOGICAL VARIABILITY

Throughout sections 1 and 2 we described procedures to quantify the level of uncertainty in baseline values, quantify the level of uncertainty in change across an intervention, and to identify if observed changes represent meaningful improvements. These procedures outlined do not, however, identify whether underlying changes occurred as a direct result of the intervention or as a result of unrelated confounding factors. Across time periods reflecting those typically used for chronic supplementation or training interventions, it is possible that an individual's true score may change due to factors external to the intervention. Take for example our 12 week hypothetical study, where $CCT_{110\%}$ was used to assess cycling capacity. High-intensity exercise performance is influenced by a wide range of factors, including nutritional intake, chronic sleep patterns and physical activity levels, with 12 weeks providing sufficient time for true scores to change in response to alterations in any of these factors. We refer to these intervention independent causes of change as *biological variability*. When combining this concept with measurement error, the potential challenges in identifying if a single individual has accrued meaningful improvements as a direct result of an intervention become clear. In section 2 of this review we outlined procedures that can be used to judge whether meaningful changes were likely to have occurred. However, these procedures do not determine the extent to which changes were the direct result of the intervention or effectively "random" external causes. For this reason, we recommend that individual responders (those that experience meaningful changes due to the direct effects of an intervention) and non-responders (those that do not experience meaningful changes due to the direct effects of an intervention) be considered as theoretical constructs that can never truly be known. Additionally, intra-individual variation in response to an intervention is rarely considered. For example, even if it were possible to establish that an individual's true score had not changed due to direct effects of an intervention (to be accurately labeled as a non-responder), it does not hold that the same result will occur if the intervention is repeated at a later time, or more conceptually, that this would be the case in each instance were it possible for the individual to complete the intervention on many occasions simultaneously. Indeed, inconsistent intra-individual changes to the same sodium bicarbonate based intervention have previously been reported, with individual analysis showing only 1 out of 15 participants improved on all four occasions above the normal variation of the test, whereas 9 out of 15 improved on at least one occasion (10). Consequently, the term *response* (and *non-response*) is preferred to indicate that in a single instance of a particular intervention, an individual has experienced (or not) a true score change caused directly by the intervention that exceeds the SWC. Given all the

challenges that exist in identifying if an individual has responded to an intervention with only a small number of data points, we concur with recent recommendations (33, 34) that researchers focus on identifying the proportion of response in group-based interventions (discussed in the following sections) or attempt to identify factors associated with response/non-response (which is considered beyond the scope of this particular review, with readers referred to Hopkins (34) for further discussion).

In the remaining sections of this review we describe procedures in group-based interventions to estimate variability in true score change directly attributable to the intervention, and, subsequently, to estimate proportion of response in a group. The procedures outlined are required during interventions with periods long enough for true score change to occur as a result of biological variability. In contrast, many nutritional supplements (e.g., caffeine or sodium bicarbonate acutely function after a single dose (35, 36) and provided the repeated tests take place within a sufficiently short time-period, then consideration of measurement error alone may be sufficient to identify proportion of response and non-response.

KEY POINTS:

- *Non-intervention related factors can often cause true scores to change. Collectively, these factors are referred to as biological variation.*
- *The terms response and non-response are used to indicate whether an individual's true score change caused by the intervention alone exceeds the smallest worthwhile change, or not, respectively.*

3.1. Estimating Variability Caused by Intervention

It is widely recognized that the most logical means of quantifying variability caused by an intervention is to include a control group or to use data from similar controls published in literature (2, 33, 34). Quantifying variation in change across a control group provides an assessment of both measurement error and biological variation. In contrast, variation in change experienced in an intervention group also accounts for the differential effects caused by the intervention. As a result, true score variation due to an intervention is equal to the change score variance of the intervention group minus change score variance of the control group. As with all concepts described in this review, variation is most useful when expressed as a standard deviation, and here we define the quantity of interest as the intervention response standard deviation (σ_{IR}). In practice, this standard deviation is estimated with the following formula $\widehat{\sigma}_{IR} = \sqrt{SD_{Int}^2 - SD_{Con}^2}$, where SD_{Int}^2 is the square of the calculated standard deviation of the observed score change from the intervention group, and SD_{Con}^2 is the square of the calculated standard deviation of the observed change scores from the control group (34). Using data from our hypothetical study as an example, MCARN standard deviations of observed score change from the control and intervention group were found to be 1.24 and 5.22 mmol·kg^{-1}DM. Note, this large difference in standard deviations measured between groups provides evidence that true change directly attributable to the intervention was highly variable across participants (33). We find that $\widehat{\sigma}_{IR} = \sqrt{SD_{Int}^2 - SD_{Con}^2} = \sqrt{5.22^2 - 1.24^2} = 5.07$ mmol·kg^{-1}DM, and as explained in the following section, this value can then be used to estimate the proportion of response.

KEY POINTS:

- *Variation in the effect of an intervention across individuals can be estimated by comparing the standard deviation of observed score change in an intervention and control group.*
- *The statistic that reflects variation in intervention effect across individuals is referred to as the intervention response standard deviation (σ_{IR}).*

3.2. Estimating Proportion of Response

Consistent with all approaches used previously in this review to estimate quantities of interest (e.g., baseline true score, true score change, or here, proportion of response), we assume a normal distribution, such that true score change directly attributable to the intervention follows a normal distribution centered on the mean observed score change, with standard deviation equal to σ_{IR} (see **Figure 6**). As the total area of any probability distribution is equal to one, the estimate of the proportion of response is obtained by calculating the area of the derived normal distribution that lies beyond the SWC (**Figure 6**). A full example calculation covering this process is included in Supplementary File (SF-S11), along with automated spreadsheet where readers can estimate proportion of response for their own datasets (SF-S12). Here we continue our example using the muscle carnosine data from our hypothetical study to provide greater clarity. The mean observed score change from the intervention group across the twelve-week period was 10.20, and as calculated in the previous paragraph, $\widehat{\sigma}_{IR} = 5.07$. Therefore, the true score change attributable to the intervention is modeled as a normal distribution with mean 10.20 mmol·kg^{-1}DM and standard deviation 5.07. If we select a SWC from standard procedures by calculating 0.2 times the baseline standard deviation, we obtain a threshold value of 2.0 mmol·kg^{-1}DM. Using the interactive calculator in the Supplementary File (SF-S11), we find that 0.947 of the area of the normal distribution described lies beyond the SWC and so we estimate that the proportion of individuals that responded to the intervention with regards to muscle carnosine content was 0.947 (i.e., we estimate that ∼95% of the supplementation group responded). This value is only an estimate, that we expect will become more accurate with greater numbers in both the intervention and control groups due to better precision in estimating $\widehat{\sigma}_{IR}$. Proportion of response is a more complex estimator than those encountered previously in the review, and confidence intervals are best derived through a resampling process such as bootstrapping. Briefly, in bootstrapping we treat our sample data as the population and repeatedly draw random samples (from the original data) each time carrying out the same calculation (e.g., proportion of response). Uncertainty in the estimate is then expressed by examining variation in the results obtained. In the Supplementary File we have included an automated spreadsheet to estimate proportion of response with the readers own data and to calculate selected confidence interval widths through bootstrapping (SF-S12). For the muscle carnosine data, the 95%

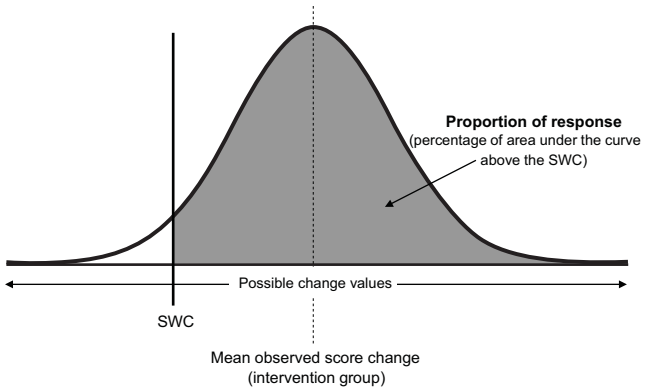

FIGURE 6 | Proportion of intervention group response. SWC, smallest worthwhile change; σ_{IR}, Intervention response standard deviation.

CI for the proportion of response estimate was found to be 88.2–100%.

KEY POINTS:

- *The different effects of an intervention across individuals can be modeled as a normal distribution centered on the mean observed score change with standard deviation σ_{IR}.*
- *Proportion of response is estimated by calculating the area of the normal distribution that lies beyond the smallest worthwhile change.*
- *Confidence intervals for the proportion of response estimate can be obtained through bootstrapping.*

Summary and Practical Recommendations

Throughout this review, we have described procedures required to interpret data collected from individuals both pre- and post-intervention. Careful and deliberate procedures are required to interpret the data appropriately, due to the fact that all measurement incorporates some degree of error (measurement error = instrumentation noise + biological noise), and changes can often occur due to factors independent of the intervention (biological variability). The procedures we have outlined enable practitioners and researchers in the area of sports nutrition to (1) establish plausible baseline values; (2) assess whether meaningful changes have occurred after an intervention; and (3) estimate the proportion of individuals in a group-based intervention that responded/did not respond to the intervention. We conclude this review with a brief summary including practical recommendations.

Prior to conducting any intervention, practitioners and researchers require baseline data to direct their choice of intervention and provide initial values to monitor and assess an intervention's progress and effectiveness. Tests and measurement procedures adopted should seek to minimize measurement error, which includes both instrumentation and biological noise. It must be recognized, however, that even when the testing environment is controlled as much as possible, some degree of measurement error will always exist. Therefore, typical error should be calculated and CIs applied to baseline measurements to provide a range of plausible true scores given the data observed. Ideally, CIs should be calculated with reliability data obtained by the practitioner using the actual equipment and procedures implemented with their clients. However, where this is not feasible, it is recommended that practitioners obtain data from published reliability studies that match their own procedures as closely as possible with regards to testing protocols and participants.

In situations where CI widths are so wide as to provide no actionable baseline information, practitioners should re-consider the specific %CI used and consider whether this can be reduced given the context of the measurement. For example, 95% CIs frequently produce large ranges for true scores and practitioners have to consider whether they require the actual true score to reside within intervals calculated in 95% of occasions. Where the safety of a client is not influenced by the intervention, narrower %CIs can be justified. For example, practitioners may choose instead to construct CIs with the observed score plus/minus the estimated TE. This calculation is simple to create and maintain across spreadsheets that practitioners may create and for baseline scores provides approximate 70% true score CIs. However, if true score intervals calculated with similar %CIs still provide limited actionable information, this suggests that the test and/or measurement processes adopted create measurement errors too large to be of practical use, and therefore an alternative and more reliable test should be considered.

Once an intervention has been completed, it is good practice to estimate true score change and provide a CI to identify a range of plausible values given the observed data. Such CIs represent the all cause change across the intervention and do not distinguish between change caused by the intervention and external factors. Where appropriate, practitioners can identify the SWC deemed to be of practical relevance for the individual, with success judged to occur when the observed score change plus/minus the estimated TE lie beyond the threshold set. In research settings, the threshold value may be set at 0, however, practitioners should select this value *a priori*. Practitioners should ensure that the estimated TE is not so large that successful interventions will frequently be deemed not-successful. To ensure this is not the case, it is recommended that practitioners identify, for example, average observed changes for specific groups of clients (which should be larger than the SWC) and make sure that TE is smaller than the difference between the average change and SWC. Where this cannot be achieved, participants will in general, be required to obtain true score changes greater than average in order for interventions to be deemed successful.

The existence of biological variability renders it challenging to isolate true score change directly caused by the intervention. For this reason, we recommend that researchers interested in this area and limited to designs with infrequent data collection (e.g., pre-intervention and post-intervention), focus at the group level and estimate proportion of response rather than attempt to identify any one individual as a responder or non-responder, and where appropriate, attempt to identify factors associated with response/non-response [see Hopkins (34) for further discussion]. To estimate the proportion of response, a control group is required, with variation between control and

intervention groups compared to quantify variation in true score change directly attributable to the intervention. An estimate of the proportion that responded can then be calculated by using the observed difference scores, standard deviations calculated from intervention and control groups, and the SWC. For all calculations and procedures suggested in this review, we have provided instructions and resources in the Supplementary File to assist.

Finally, it is important to acknowledge the differences between combining procedures outlined to identify an intervention successful for an individual (e.g., true score change CI's and SWC, as demonstrated in SF-S9), and estimating the proportion of response in group-based interventions (SF-S11). With the former, there is no attempt to distinguish between intervention and non-intervention causes of change. In addition, the procedures outlined for the individual are heavily influenced by the relative magnitudes of measurement error and SWC. The approach described herein, requires that an individual's observed score change exceeds the SWC by, at least, the TE of the test. In scenarios where the TE is large, individuals will typically require true score changes substantially beyond SWC to identify an intervention as a success. Note, this conservative approach is required to routinely avoid individuals obtaining observed score changes greater than the SWC due to the randomness of measurement error alone. In contrast, the procedures described in section 3 to estimate proportion of response do distinguish between intervention and non-intervention causes of change. Estimating the proportion of response using this approach, is to some extent, less influenced by large measurement errors. This is due to the fact that the effects of measurement error are accounted for by variation observed in the control group and are thus removed from the final calculation. With greater participant numbers in the intervention and control group, estimates will become more precise and uncertainty reduced. As a result of these differences, it is possible that the proportion of individuals identified to experience a successful intervention (SF-S9), and the estimate of the proportion of response (SF-S11) will be different. Given the infrequent data collection points routinely used in practice (e.g., pre- and post-intervention), caution is required when interpreting at the level of individuals and it should be remembered that CI's are to be interpreted over the long-run. In scenarios where large measurement errors occur, practitioners/researchers can use knowledge of group-based estimates of response, to provide greater context when evaluating data observed from individuals.

CONCLUSION

A personalized approach to sports nutrition is increasing in popularity due to recognition of the myriad of factors that influence individual response to nutrition and exercise related interventions. The presence of measurement error and biological variation renders identification of baseline values, change values and response status challenging, thus strategies to account for these issues have been proposed, enabling practitioners, and researchers to make informed decisions and judgements from the data they collect.

AUTHOR CONTRIBUTIONS

ED and PS originally conceived the idea for this review. PS provided the statistical expertise and lead the writing of the review, along with the development of the Supplementary Files, with support from BH. Ongoing critical input was received from BS, BG, and ED. 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/fnut.2018.00041/full#supplementary-material

A maintained version of this Supplementary File can be found at github.com/sportscientist.

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Conflict of Interest Statement: 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.

4. APPENDIX I

For the calculation of CIs it is useful to introduce additional notation and concepts. The first is the notation: $100\,(1-\alpha)\,\%$, which describes the width of the CI. Here, α is a variable that we choose to set the interval and importantly link the width to the correct multiple of our TE estimate. For example, to set a 90% CI then α must be set to $\alpha = 0.1$ to give $100\,(1-0.1)\,\% = 90\%$. Given the consistent assumptions that observed scores are normally distributed we evoke the relevant properties of the distribution, such that a $100\,(1-\alpha)\,\%$ CI for true score is obtained with $O_s \pm TE \times Z_{(1-\alpha/2)}$. The coefficient $Z_{(1-\alpha/2)}$ is referred to as the $(1-\alpha/2)$-th quantile of the standard normal distribution. In our example where we set α to 0.1 (i.e., for a 90% confidence interval), we require $Z_{(1-0.1/2)}$, or the 0.95th quantile of the standard distribution. To obtain this value we can look up standard statistical tables or use software such as MS Excel. Using these methods, we find that $Z_{0.95}$ is equal to 1.64 and so a 90% true sore CI for an individual would equal $O_s \pm TE \times 1.64$.

It is important to acknowledge that we can never definitively state the TE and studies only report imperfect estimates $\widehat{\text{TE}}$, where accuracy will depend primarily on the number of individuals (or number of repeated trials) used in a test-retest. To account for this additional uncertainty, we use the $(1-\alpha/2)$-th quantile value from a t-distribution which is similar in shape to the normal distribution but has heavier tails (i.e., greater proportion of values away from the center). The specific t-distribution is based on numbers used in our TE estimate and we say that it has degrees of freedom equal to $n-1$. In the data sets provided in this review, we include 20 participants ($n = 20$) to estimate TE from test-retests, and as such a 90% true score CI for each individual is equal to $O_s \pm \widehat{\text{TE}} \times t_{19,\,0.95}$, (i.e., the 0.95th quantile of the t-distribution with 19 degrees of freedom). Looking up statistical tables or use of software identifies that $t_{19,\,0.95} = 1.73$ and so our 90% true score CI is calculated with $O_s \pm \widehat{\text{TE}} \times 1.73$. Alternatively, if we wanted to calculate a 50% true score CI with the t-distribution, we would set $\alpha = 0.5$, $t_{19,\,0.75} = 0.69$ to give $O_s \pm \widehat{\text{TE}} \times 0.69$. What is important to note, is that as the number of individuals increases the t-distribution approaches the normal distribution such that the coefficients used to multiply the TE become similar.

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