International Journal of Sport Nutrition and Exercise Metabolism, 2024, 34, 1-10
https://doi.org/10.1123/ijsnem.2023-0078

Human Kinetics
ORIGINAL RESEARCH

Effects of Ketone Monoester and Bicarbonate Co-Ingestion on Cycling Performance in WorldTour Cyclists

Domingo Jesús Ramos-Campo,[1] Francisco Javier López-Román,[2,3] Silvia Pérez-Piñero,[2] Raquel Ortolano,[2] María Salud Abellán-Ruiz,[2] Enrique Molina Pérez de los Cobos,[2,3] Antonio Jesús Luque-Rubia,[2] Dag Van Elslande,[4] and Vicente Ávila-Gandía[2]

[1]LFE Research Group, Department of Health and Human Performance, Faculty of Physical Activity and Sport Science-INEF, Universidad Politécnica de Madrid, Madrid, Spain; [2]Department of Sports Physiology, Faculty of Health Sciences, UCAM Universidad Católica San Antonio de Murcia, Murcia, Spain; [3]Primary Care Research Group, Biomedical Research Institute of Murcia (IMIB-Arrixaca), Murcia, Spain; [4]Sports Medicine, Waregem, Belgium

The present randomized study investigated the effect of acute supplementation of 800 mg/kg of ketone monoester ingestion (KE) or placebo (PL) and 210 mg/kg of $NaHCO_3$ co-ingestion on cycling performance of WorldTour cyclists during a road cycling stage simulation. Twenty-eight cyclists participated in the study (27.46 ± 4.32 years; 1.80 ± 0.06 m; 69.74 ± 6.36 kg). Performance, physiological, biochemical, and metabolism outcomes, gut discomfort, and effort perceived were assessed during a road cycling simulation composed of an 8-min time-trial (TT) performance + 30-s TT + 4.5 hr of outdoor cycling + a second 8-min TT + a second 30-s TT. Greater absolute and relative mean power during the first 8-min TT ($F = 5.067$, $p = .033$, $\eta_p^2 = .163$, $F = 5.339$, $p = .029$, $\eta_p^2 = .170$, respectively) was observed after KE than after PL (KE: 389 ± 34, PL: 378 ± 44 W, $p = .002$, $d = 0.294$ and KE: 5.60 ± 0.42, PL: 5.41 ± 0.44 W/kg, $p = .001$, $d = 0.442$). Additionally, greater concentration of β-hydroxybutyrate blood concentration ($F = 42.195$, $p < .001$, $\eta_p^2 = .619$) was observed after KE than after PL during the first steps of the stage (e.g., after warm-up KE: 1.223 ± 0.642, PL: 0.044 ± 0.058 mM, $p < .001$, $d = 2.589$), although the concentrations returned to near baseline after 4.5 hr of outdoor cycling. Moreover, higher values of anion gap were observed ($F = 2.333$, $p = .026$, $\eta_p^2 = .080$) after KE than after PL ingestion, after the warm-up and after the first 8-min and 30-s TT. Additionally, lower concentrations of HCO_3^- were reported in the KE condition after warm-up and after the first 8-min and 30-s TT. During the initial phase of the stage simulation, acute supplementation with KE + $NaHCO_3$ co-ingestion enhanced 8-min TT cycling performance (3.1%) in WorldTour cyclists with a concomitant hyperketonaemia.

Keywords: acid–base status, ketosis, supplementation, time trial

Carbohydrates are the primary fuel source during moderate- to high-intensity efforts in road cycling (Burke & Hawley, 2018; Kerksick et al., 2017) but their availability is limited by glycogen stores, blood glucose, and lactate levels. Cyclists often employ nutritional strategies to spare glycogen (Burke & Maughan, 2015). Ketone supplementation has been suggested as a complementary approach to reduce carbohydrate reliance and promote glycogen sparing during exercise (Evans et al., 2022). Ketone bodies (KB) can serve as energy substrates for various tissues, including skeletal muscles and the heart, with greater contribution observed in exogenous ketosis (Evans et al., 2017). Hyperketonemia is defined as a plasma ketone concentration above 0.5 mM (Robinson & Williamson, 1980). β-Hydroxybutyrate (βHB) is the most common circulating ketone body, and exogenous ketones are often formulated as βHB monoesters (Brooks et al., 2022; Harvey et al., 2019).

Previous studies reported mechanistic effects of KB, finding that they inhibit glycolytic flux (Maizels et al., 1977), obtaining an extra energy supply while sparing muscle glycogen stores (Evans et al., 2017). Additionally, when KB are used as a substrate, more efficient adenosine triphosphate (ATP) production is observed, as compared with glucose or fatty acids due to a greater free energy released from ATP hydrolysis and less oxygen required per mole of carbon (Egan & D'Agostino, 2016; Evans et al., 2022). Moreover, an original study (Cox et al., 2016) reported that KB elicit a reduced reliance on carbohydrate utilization during exercise, reducing glycolytic rates and lactate accumulation during moderate- to high-intensity exercise. Conversely, ketone ingestion leads to metabolic acidosis, reducing blood pH and alkaline reserve (Poffé, Wyns, et al., 2021), which is linked to fatigue. When the pH is slightly decreased, as observed after ketone ingestion (Dearlove et al., 2019), fatigue perception increases and exercise performance is decreased (Poffé, Wyns, et al., 2021) by extracellular acidosis (Carr et al., 2011). Therefore, the literature on ketone research speculates that oral ketone ingestion might enhance endurance, but this beneficial metabolic effect may be nullified by the appearance of ketosis-induced metabolic acidosis (Dearlove et al., 2019), and glycolytic inhibition (Cox et al., 2016), which may negatively affect exercise performance.

Supplementation with $NaHCO_3$ improves performance in high-intensity cycling reducing acid-base disturbances (Grgic et al., 2021) being a common ergogenic aid in cycling, specifically in time-trial (TT) stages. Recently, one study (Poffé, Wyns, et al., 2021) found that the co-ingestion of 65 g of ketone monoester (KE) and 300 mg/kg of $NaHCO_3$ maintained the acid-base balance, thus avoiding ketoacidosis and increasing cycling performance. However, the same research group has also published a study showing exogenous ketosis (50 g of KE) impairs 30-min TT performance independent of bicarbonate supplementation (180 mg/kg; Poffé, Wyns, et al., 2021). Therefore, equivocal results have been reported, and the topic is still under debate. In this way, an ergogenic effect of exogenous KE on performance has been found by some previous studies on cycling (Cox et al., 2016;

Pérez-Piñero https://orcid.org/0000-0002-2027-9075
Ramos-Campo (domingojesusramos@gmail.com) is corresponding author, https://orcid.org/0000-0002-8890-4244

Poffé, Wyns, et al., 2021) but other studies have demonstrated no effects on running (Evans et al., 2019; Evans & Egan, 2018) or even showing a decrease in cycling performance (Poffé, Wyns, et al., 2021). In this context, some recent meta-analysis (Brooks et al., 2022; Margolis & O'Fallon, 2020; Valenzuela et al., 2020) reported no significant improvements on endurance exercise performance after exogenous ketone administration.

The training level of the participants has been also argued as an outcome that affects the effect of exogenous ketone ingestion (Evans et al., 2022). Specifically, the oxidation of KB as an alternative substrate is influenced by the adaptations promoted by exercise, which maximize the transport of the substrates, produce a greater the capacity of KB uptake (Ohmori et al., 1990), and increase the concentrations of ketolytic enzyme in the skeletal muscle (Winder et al., 1974). Therefore, previous studies proposed that the effect of KB as an alternative substrate for energy provision is likely to be greatest in highly endurance-trained individuals (Evans et al., 2022), but this hypothesis only has preliminary evidence in humans (Dearlove et al., 2021). Hence, it is necessary to investigate the effect of exogenous ketone + $NaHCO_3$ co-ingestion in high endurance-trained athletes (e.g., WorldTour cyclist), to confirm this previous hypothesis. Conversely, previous studies lack real-world application and scarce ecological designs simulate road cycling stages in WorldTour cyclists. Therefore, this study sought to analyze (a) the direct effect of acute supplementation of KE or placebo (PL), along with $NaHCO_3$ co-ingestion, on cycling performance in WorldTour cyclists during the initial stages of a road cycling stage simulation and (b) the indirect effect of the same supplementation on cycling performance in cyclists during the later stages of the event. Our hypothesis was that the supplementation of ketone + $NaHCO_3$ would lead to an improvement in cycling performance, both in the early and later stages of the event, compared with the supplementation of PL + $NaHCO_3$ in WorldTour cyclists.

Methods

Design

A double-blind, randomized and comparative crossover study was conducted to analyze the effect of KE and $NaHCO_3$ co-ingestion or PL and $NaHCO_3$ co-ingestion on cycling performance in WorldTour cyclists (Figure 1). The testing sessions took place during the first training camp of the season of the team. The trial was conducted following the principles of the Declaration of Helsinki and received approval from the local Science Ethics Committee (identifier: CE112105). Additionally, it was preregistered in clinicaltrials.gov (NCT05294939).

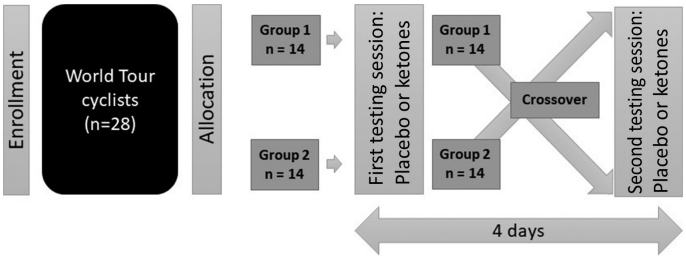

Figure 1 — Research design. The study involved two experimental sessions in a double-blind, randomized, crossover design. At each condition, cyclists ($n = 29$) ingested ketone monoester or placebo and $NaHCO_3$ co-ingestion during a road cycling stage simulation.

Participants

Twenty-nine male WorldTour cyclists from a Union Cycliste Internationale World Team were initially enrolled in this study. However, one participant was excluded due to an exclusion criterion. Therefore, 28 cyclists (age: 27.46 ± 4.32 years, height: 1.80 ± 0.06 m, weight: 69.74 ± 6.36 kg, fat mass: 6.98 ± 2.27%, VO_2max = 70.8 ± 4.7 $ml \cdot kg^{-1} \cdot min^{-1}$) were included in the final analysis. Cyclists signed an informed consent form before starting the study. The inclusion criteria of the study were: (a) male professional cyclist belonging to a Union Cycliste Internationale World Team and (b) no intense training the day before the study. Cyclists were excluded if (a) had an injury during the month prior to the study that modified their training load, (b) used chronic supplementation of ketones during the month prior to the study, and (c) ingested other performance-enhancing aids during the study.

Supplementation Protocol

Cyclists randomly ingested (R)-3-hydroxybutyl (R)-3-hydroxybutyrate KE (KE4, KetoneAid Inc.), or a matching PL containing denatonium benzoate in a beverage similar in appearance, color, and smell, stored in opaque bottles in a typical double-blind trial. A simple randomization was performed by a scientist not participating in the study, using software (Epidat 4.2) that generated random codes which were assigned to participants with a 50% chance of ingesting the KE or PL. The blinding efficacy was verified after the participants had finished their participation. Both treatments involved the consumption of using two boluses: (a) 30 min before warm-up and (b) 90 min after the first serving. The total amount of KE was 800 mg/kg, following the protocol reported previously (Poffé, Ramaekers, et al., 2021; 922 ± 85 mg/kg), which promoted a beneficial effect of Ketone + $NaHCO_3$ co-ingestion in cycling performance. The last meal was eaten by the cyclist 3 hr before the testing session (1,651 Kcal [24.00 ± 2.11 Kcal/kg], 61.3% carbohydrates [3.68 ± 0.31 g/kg], 25.62% fat [0.69 ± 0.06 g/kg], and 13.08% protein [0.78 ± 0.08 g/kg]). Diet was monitored during the entire training camp, and participants followed a similar diet in the 48 hr before each treatment session: (a) day before Test 1: 3,437.8 Kcal (50.00 ± 4.40 Kcal/kg); 56.94% carbohydrate (7.12 ± 0.62 g/kg), 23.56% fat (1.30 ± 0.11 g/kg), and 18.73% protein (2.35 ± 0.21 g/kg), (b) day before Test 2: 3,707.85 Kcal (53.90 ± 4.75 Kcal/kg); 55.56% carbohydrate (7.50 ± 0.65 g/kg), 25.97% fat (1.56 ± 0.14 g/kg), and 18.02% protein (2.44 ± 0.21 g/kg). Additionally, 30 min before exercising to the end of the testing session, the cyclists ingested 90 $g \cdot hr^{-1}$ of carbohydrates (Hydrafit, Energybar and Totalenergy gel, Namedsport SRL.). In addition, the cyclists of the present study used to include bicarbonate as ergogenic aid in competition. Therefore, the present study the supplementation in both groups included bicarbonate in order to simulate the most real-world road cycling race. Specifically, the participants received 30 mg/kg of $NaHCO_3$ using enteric-coated tablets (Bicanorm, Fresenius Medical Care AG) in the following time points: (a) after breakfast, (b) 90 min before the testing session, (c) at the start of the testing session, (d) every 60 min during the testing session.

Testing Session

Two weeks before the stage, cyclists conducted an incremental test until exhaustion with gas analysis. The test included a 5-min warm-up at 150 W, followed by an increase of 30 W/min starting at 180 W. On the day of the testing session, the cyclists arrived to the

testing zone and their body composition was assessed using a bioimpedance segmental analyzer (Tanita BC-601, Tanita Corp). A capillary tube of 65 µL was used to collect a blood sample to establish the baseline concentrations of the following metabolites and acid-base status parameters (ABL 90 Flex, Radiometer): pH, bicarbonate (HCO_3; mM), anion gap (mEq/L), blood lactate (mM), and glucose (Glu; mM). In addition, βHB concentrations (mM) were assessed using the FreeStyle Optium Neo Meter25 device (Abbott Diabetes Care). Gut discomfort was evaluated using a visual analogic scale (from 0 mm [*none discomfort*] to 100 mm [*maximum discomfort*]). After the baseline measurements, the participants ingested the first bolus of the KE or PL drink, and 30 min later, a second evaluation was performed prior the warm-up. The cyclists performed their traditional TT warm-up that lasted 10 min, including cycling at low-intensity and progressive cycling sets. All participants were familiarized with the testing sessions because they performed similar testing during the season. The testing session was set-up to simulate the characteristics of a road cycling stage, where the first kilometers usually include high-intensity efforts to attack and breakaway from other riders, after which the stage usually includes some hours of continuous moderate-intensity effort, and finally, during the last kilometers to find the winner, the stage includes high-intensity efforts and sprints. Cycling performance was the primary outcome.

The testing session was developed in the following manner: when the cyclists finished the warm-up and after the third blood sample collection, they performed an 8-min TT followed by a 30-s sprint test with 3 min of rest between them. The TTs were performed by putting the participant's own bicycle on the Home-trainer Tacx Neo 2T Smart trainer (Tacx Inc). Power was assessed by a power meter (Shimano DURA-ACE FC-R9200-P, Shimano Inc.) and a cycling computer (Garmin Edge 1040, Garmin Inc.) during all the testing sessions. Additionally, heart rate (Garmin HRM-Pro chest strap, Garmin Inc.) and rating of perceived exertion using the Borg CR10 scale, were assessed. Two more blood samples were collected at the end of each TT.

After this first part of the testing session, cyclists biked outdoors for 4.5 hr with 2,000 m of positive accumulation, simulating a road stage. During the cycling session, 90 min after the first bolus ingestion, the participants ingested the second bolus of KE or PL. When the simulation of the stage ended, the cyclist arrived to the testing zone and performed a second 8-min TT followed by a 30-s test with the previously described protocol. The mean environmental conditions during the first and second testing session were of 15.7 ± 2.6 °C and 64.6 ± 4.4% relative humidity and 14.8 ± 2.3 °C and 69.7 ± 3.8% relative humidity, respectively.

The same procedure was applied 4 days later, but the cyclists ingested the second randomized treatment (Figure 2).

Statistical Analysis

The statistical analysis of the data was performed using the Statistical Package for Social Sciences software (version 26.0, SPSS Inc.). Descriptive analyses were calculated. For inferential analysis, a Shapiro–Wilk *W* test was performed to establish the normality of the sample distribution, and a Mauchly's *W* test analyzed the sphericity between measurements. For the treatment in which the sphericity assumption was not met, the Greenhouse–Geisser sphericity criterion was applied. In addition, a repeated-measures analysis of variance (Time × Treatment) was performed (general linear model) to analyze the effects of KE on cycling performance. Post hoc tests (Bonferroni) were performed when significant interaction effects were observed. A paired samples *T* test was used to compare the effect of KE on the road cycling testing session and the order effect. In addition, the sample size was calculated based on the mean power in the first 8-min TT, which served as the main outcome of the study. Considering a *SD* of the mean power of 12.3 W reported in a similar population (Ávila-Gandía et al., 2021), a precision of 9 W with an alpha risk of 5%, and a statistical power of 80%, 23 participants are required. Accounting for a potential 25% loss to follow-up, a total of 29 cyclists were recruited. Effect size calculated using partial eta-squared (η_p^2) for variance analysis and as Cohen's *d* for pairwise comparisons. Cohen's *d* effect sizes were interpreted using thresholds of <0.25, ≥0.5, and ≥1.0 for trivial, small, medium, and large, respectively (Rhea, 2004). The smallest worthwhile change (SWC) was set at 0.2 between-subject *SD*, which is suggested to represent a practically relevant change in performance in cyclists. Thus, the

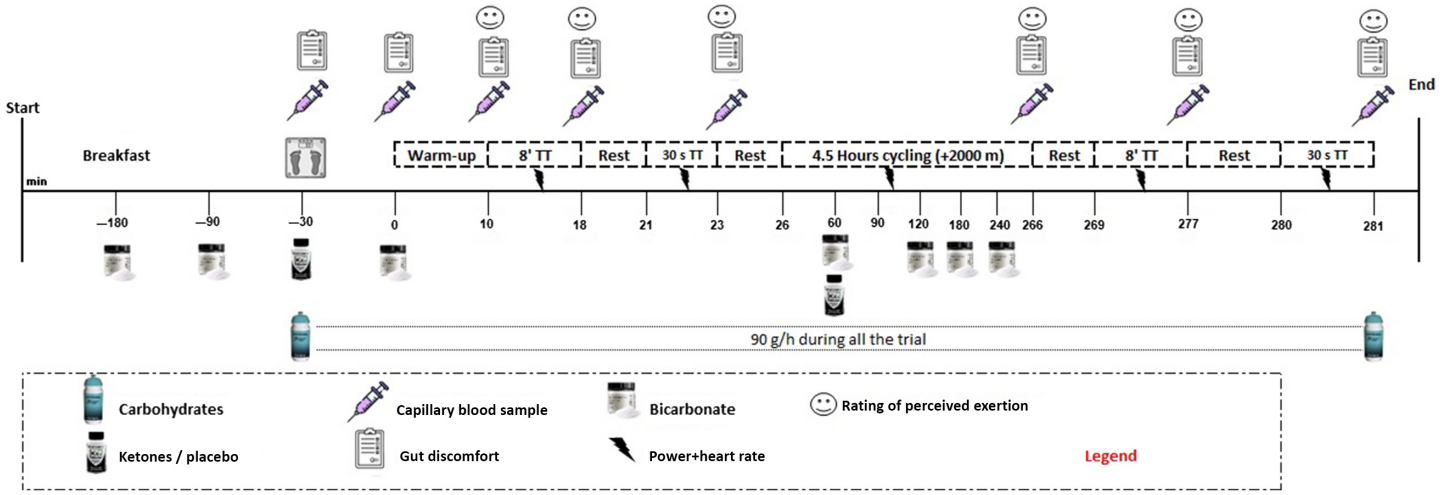

Figure 2 — Schematic representation of the testing sessions. At each experimental condition (KE + $NaHCO_3$ or PL + $NaHCO_3$), cyclists ($n = 29$) performed a real field road cycling stage simulation composed of an 8-min TT performance + 30-s TT + 4.5 hr of outdoor cycling + a second 8-min TT + a second 30-s TT. KE = ketone monoester; PL = placebo; TT = time trial.

SWC corresponded to 8.8 W for 8 min TT performance in this study. Correlations between VO_2max, βHB concentration, and performance were tested using Pearson's test. For all procedures, the level of significance was set at $p \leq .05$.

Results

With respect to the performance variables (Table 1 and Figures 3), a main effect was found in absolute and relative mean power during the first 8-min TT ($F = 5.067$, $p = .033$, $\eta_p^2 = .163$ and $F = 5.339$, $p = .029$, $\eta_p^2 = .170$, respectively) showing greater performance after KE than after PL (absolute mean power KE: 389 ± 34, PL: 378 ± 44 W, $p = .002$, $d = 0.294$ and relative mean power KE: 5.60 ± 0.42, PL: 5.41 ± 0.44 W/kg, $p = .001$, $d = 0.442$). Specifically, 14 participants obtained a greater improvement than the SWC (8.8 W) during the first 8-min TT after KE (Figure 4). However, no main effect was found on other performance and heart rate outcomes. No significant differences were observed in performance, training, and physiological outcomes measured during the 4.5-hr outdoor cycling session (Table 2). Nevertheless, no main effect was found in rating of perceived exertion and gut discomfort (Table 3).

Regarding the acid-base and metabolic variables (Figure 5), a main effect was found on βHB ($F = 42.195$, $p < .001$, $\eta_p^2 = .619$), observing a greater concentration in the KE treatment than in the PL one, before (KE: 1.128 ± 0.935 [range = 0.1–3.30 mM], PL: 0.047 ± 0.078 mM, $p < .001$, $d = 1.629$) and after warm-up (KE: 1.223 ± 0.642 [range = 0.1–2.30 mM], PL: 0.044 ± 0.058 mM, $p < .001$, $d = 2.587$), and after the first 8-min (KE: 1.111 ± 0.627 [range = 0.1–2.30 mM], PL: 0.085 ± 0.082 mM, $p < .001$, $d = 2.295$), and 30-s (KE: 1.025 ± 0.577 [range = 0.1–2.20 mM], PL: 0.102 ± 0.041 mM, $p < .001$, $d = 2.254$) TTs. No differences were observed in baseline, nor after the 4.5 hr of the simulated road stage, or after the second 8-min and 30-s TT, as the βHB concentrations returned to values close to baseline values after the 4.5 hr of cycling. In addition, no correlation was found between mean power during the first 8-min TT and βHB ($r = .366$, $p = .112$) nor VO_2max ($r = .191$, $p = .420$). Moreover, an effect was observed on the anion gap (mEq/L; $F = 2.333$, $p = .026$, $\eta_p^2 = .080$) showing greater values after KE than after PL ingestion after the warm-up (KE: 11.92 ± 2.03, PL: 8.84 ± 3.51 mEq/L, $p < .001$, $d = 1.074$) and after the first 8-min (KE: 21.42 ± 3.58, PL: 18.99 ± 5.33 mEq/L, $p = .027$, $d = 0.535$) and 30-s TT (KE: 24.48 ± 4.03, PL: 22.16 ± 4.98 mEq/L, $p = .041$, $d = 0.512$). A treatment effect was observed on HCO_3^- ($F = 10.650$, $p = .003$, $\eta_p^2 = .283$), showing lower concentrations in the KE treatment after warm-up (KE: 23.43 ± 2.63, PL: 26.21 ± 4.03 mM, $p = .006$, $d = 1.109$) and after the first 8-min TT (KE: 13.62 ± 2.50, PL: 15.50 ± 3.89 mM, $p = .013$, $d = 0.575$) and 30-s TT (KE: 12.34 ± 3.01, PL: 14.62 ± 5.26 mM, $p = .013$, $d = 0.532$). However, no main effects were found in pH, glucose, and lactate.

After the final experimental session, cyclists were asked to identify the order of their experimental conditions. Of the 28 cyclists, 21 (72%) declared that they could not differentiate between PL + $NaHCO_3$ and KE + $NaHCO_3$, and eight (28%) of the participants was able to correctly identify the two experimental conditions indicating successful blinding.

Finally, there was no order effect of the testing session on performance outcomes (relative mean power during the first 8-min TT: first session: 5.46 ± 0.44, second session: 5.55 ± 0.44 W/kg, $p = 0.168$, $d = 0.205$; relative mean power during the first 30-s TT: first session: 9.22 ± 1.19, second session: 9.40 ± 0.83 W/kg, $p = .346$, $d = 0.175$; relative mean power during the second 8-min TT: first session: 5.41 ± 0.44, second session: 5.38 ± 0.51 W/kg, $p = .584$, $d = 0.063$; and relative mean power during the second 30-s TT: first session: 8.73 ± 1.05, second session: 8.93 ± 1.24 W/kg, $p = .230$, $d = 0.174$).

Discussion

This is the first study to report the effect of acute supplementation with KE or PL and $NaHCO_3$ co-ingestion on cycling performance during a simulated road cycling stage in WorldTour cyclists who undertook similar road race nutritional strategies (i.e., prerace and race competition diet). The main finding of the present study was that acute supplementation of KE + $NaHCO_3$ co-ingestion, improved cycling performance during the first 8-min TT (3.1%) with a concomitant occurrence of hyperketonemia. However, no significant changes in cycling performance were observed during the end phase of the stage simulation in the WorldTour cyclists. Therefore, our initial hypothesis was partially fulfilled. Although the supplementation utilized produced ketoacidosis after the first

Table 1 Performance Results During the Study After Ketones or Placebo Ingestion

				Time × Treatment			
Outcome	**Treatment**	**First TT**	**Second TT**	***F***	***p***	**η_p^2**	**Comparison**
Mean power 8-min TT (W)	Ketones	389 ± 34	377 ± 38	5.067	.033	.163	Ketones vs. placebo first TT, $p = .002$; $d = 0.294$
	Placebo	378 ± 44	377 ± 40				Ketones first vs. second, $p = .003$; $d = 0.349$
Mean power 8- min TT (W/kg)	Ketones	5.60 ± 0.42	5.40 ± 0.48	5.339	.029	.170	Ketones vs. placebo first TT, $p = .001$; $d = 0.442$
	Placebo	5.41 ± 0.44	5.39 ± 0.47				Ketones first vs. second, $p = .001$; $d = 0.443$
Mean heart rate 8-min TT (bpm)	Ketones	170.21 ± 9.32	167.24 ± 11.48	3.520	.072	.119	Ketones vs. placebo first TT, $p = .033$; $d = 0.322$
	Placebo	166.89 ± 11.21	166.91 ± 13.02				
Mean power 30-s TT (W)	Ketones	652 ± 76	619 ± 96	0.007	.932	.000	Ketones first vs. second, $p = .012$; $d = 0.386$
	Placebo	649 ± 114	617 ± 105				Placebo first vs. second, $p = .012$; $d = 0.294$
Mean power 30-s TT (W/kg)	Ketones	9.36 ± 0.85	8.86 ± 1.14	0.046	.832	.002	Ketones first vs. second, $p = .011$; $d = 0.497$
	Placebo	9.26 ± 1.18	8.80 ± 1.16				Placebo first vs. second, $p = .012$; $d = 0.393$

Note. TT = time trial.

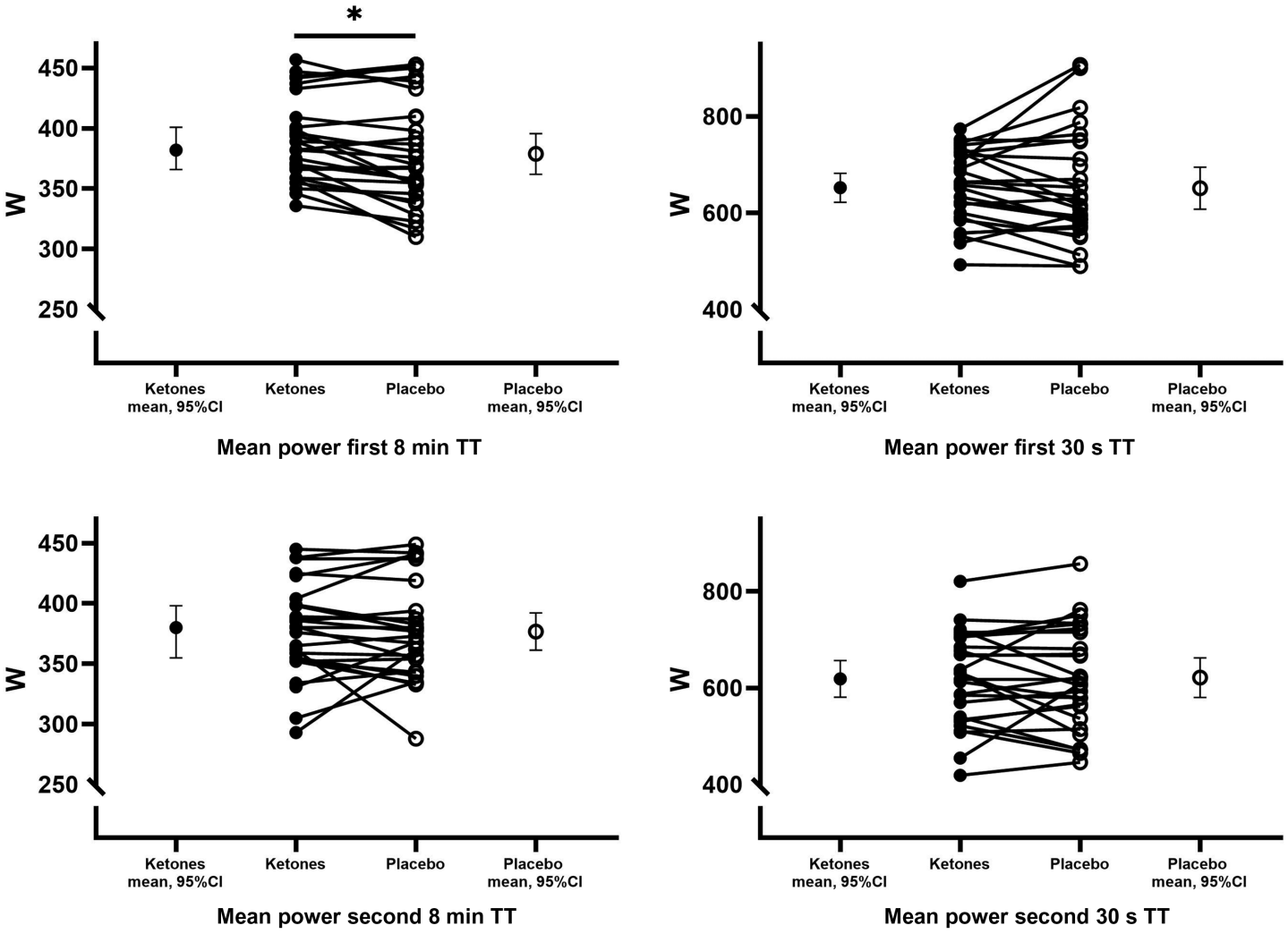

Figure 3 — Effect of $KE + NaHCO_3$ or $PL + NaHCO_3$ supplementation on exercise performance. Data are mean ± *SD* and the individual values of mean power of the first 8-min TT, mean power of the second 8-min TT, mean power of the first 30-s TT, and mean power of the second 30-s TT. *Significant differences between ketones and PL, $p \leq .05$. KE = ketone monoester; PL = placebo; TT = time trial.

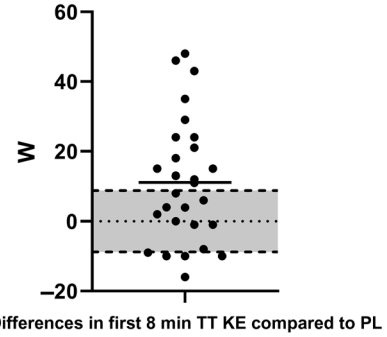

Figure 4 — Differences in the first 8-min TT performance between $KE + NaHCO_3$ compared with $PL + NaHCO_3$. The shaded area in represents the range for the smallest worthwhile difference in first 8-min TT performance in this cohort. KE = ketone monoester; PL = placebo; TT = time trial.

bolus ingestion, there was a modest change in the average concentration of βHB. It is further suggested that this change is expected to be even smaller after the administration of a second bolus of βHB. Consequently, based on these findings, it is not surprising that there were no significant effects observed in relation to blood, gut, or performance outcomes in the second 8-min and 30-s TT.

The beneficial effects of exogenous ketones on endurance are still under debate. Some studies have reported an impairment in performance during high-intensity cycling (Leckey et al., 2017; McCarthy et al., 2023; O'Malley et al., 2017; Poffé, Wyns, et al., 2021). Conversely, other trials found no beneficial effects after acute ketone supplementation in cycling during a 15-min TT at the end of a 3 hr and 15 min simulated cycling race (Poffé et al., 2020), or 10-km running (Evans et al., 2019). However, a study by Cox et al. (2016) found that exogenous ketone ingestion resulted in an ergogenic effect of 2% during a 30-min TT following 60 min at 75% of peak power. In line with the current debate, our study's findings demonstrate a positive impact on cycling performance in the initial phase of a simulated road cycling stage. Specifically, among the participants, 50% achieved a greater improvement

Table 2 Performance Results During the 4.5 hr of Outdoor Cycling After Ketones or Placebo Ingestion

Outcome	Ketones	Placebo	*t*	*p*	Effect size (*d*)
Distance (km)	140.99 ± 5.51	140.19 ± 4.82	.580	.567	0.155
Duration (min)	286.72 ± 16.12	287.96 ± 17.78	.590	.561	0.073
Mean speed (km/hr)	31.41 ± 0.62	31.36 ± 0.64	.589	.561	0.079
Mean power (W)	195 ± 15	194 ± 15	.432	.669	0.046
Relative mean power (W/kg)	2.80 ± 0.17	2.79 ± 0.16	.593	.558	0.061
Cadence (rev/min)	78.98 ± 3.23	79.07 ± 2.87	.284	.779	0.029
Vertical meters (m)	2,229.73 ± 138.12	2,209.52 ± 123.37	.870	.393	0.154
Intensity factor (a.u.)	0.97 ± 0.07	0.97 ± 0.08	.509	.616	0.000
Mean heart rate (beats/min)	130.49 ± 7.59	129.44 ± 7.10	1.633	.115	0.143
Training stress score (a.u.)	476.94 ± 25.09	479.05 ± 28.18	.601	.554	0.079

Note. a.u. = arbitrary units; rev = revolution.

(3.1%) than the SWC (8.8 W) during the first 8-min TT following the consumption of KE. Furthermore, a more recent study by Poffé, Wyns, et al. (2021) showed an improvement of 5% in mean power during a 15-min TT at the end of 3 hr of a simulated cycling race, after a concomitant ingestion of KE + $NaHCO_3$, in order to counteract ketoacidosis. Factors such as the duration of the activity, the specific protocol used, and the timing and amount of carbohydrate ingestion can have an impact on the observed effects.

In the present study, the improvement in performance was accompanied by hyperketonemia following the ingestion of KE, as evidenced by the reported blood βHB concentrations (~1.2 mM after the first bolus). The higher concentration of KB in the blood, which serves as a substrate, has been linked to more efficient ATP production compared with glucose or fatty acids. This increased efficiency is attributed to a greater release of free energy during ATP hydrolysis and a reduced oxygen requirement per mole of carbon (Egan & D'Agostino, 2016; Evans et al., 2022). These potential mechanistic effects of KB can help explain the observed enhanced performance following KE. However, despite the ingestion of a second KE bolus 90 min after the initial one, the blood βHB concentration returned to baseline values by the time the cyclists completed the 4.5 hr of outdoor cycling. It is worth noting that the duration of hyperketonemia achieved by the participants remains unclear, as blood sampling was not feasible during the 4.5 hr of outdoor cycling. The decrease in blood βHB levels below ketoacidosis thresholds (0.5 mM) could potentially explain the absence of performance effects in the final stage of the event.

In the present study, a ketone dose of 800 mg/kg was administered. Previous studies using similar dosages of 726 mg/kg found a detrimental effect (Poffé, Wyns, et al., 2021) or no effect on performance using 573 mg/kg of KE (Evans et al., 2019). Conversely, it is worth noting that previous studies reporting performance enhancements have utilized higher dosages of 922 mg/kg (Poffé, Wyns, et al., 2021) and lower dosages of 573 mg/kg (Cox et al., 2016) than in the current study. However, the results regarding the relationships between the administered dosage and blood ketone concentration are controversial. Previous studies reporting performance improvements (Cox et al., 2016; Poffé, Wyns, et al., 2021) reported βHB concentrations < 2 mM after 922 mg/kg and 573 mg/kg of KE ingestion, respectively. However, in the present study, the βHB concentrations after the administration of 800 mg/kg of KE were lower (~1.2 mM) than in these aforementioned studies. These findings align with Leckey et al. (2017), who found a modest increase of 0.3 mM in serum βHB concentrations after 500 mg/kg of ketone diester ingestion in WorldTour cyclists.

Interestingly, the current study found a substantial interindividual response to KE ingestion, with maximum blood βHB concentrations ranging from 0.1 to 3.3 mM after the ingestion of the first bolus. This novel finding is absent in the existing literature, possibly due to the utilization of smaller sample sizes in previous studies, in contrast to the present investigation involving 28 WorldTour cyclists. These divergences in βHB concentrations are likely explained by a range of factors (Leckey et al., 2017), including the different ketone esters used, the elite training status of cyclists in the current study, and the different preingestion nutritional strategies where the current study focused on appropriate race preparation practices. Therefore, it appears that multiple factors could potentially influence the effects of acute ketone ingestion, leading to heterogeneity in plasma concentration. Hence, the results obtained in the present study may not be directly attributable to the dosage or blood ketone concentration. Further work is required to analyze the factors that impact ketosis and to determine the mechanisms contributing to this effect.

The ingestion of ketones may influence performance by promoting acid-base disturbances during exercise (i.e., ketoacidosis and decrease of alkaline reserve [HCO_3^-]; Dearlove et al., 2019; Poffé et al., 2020; Poffé, Wyns, et al., 2021; Stubbs et al., 2019) that could explain some findings of the present research. In the current study, pH changes were similar in both treatments. However, to maintain a similar pH, a higher activity of the buffering capacity by the decrease of blood HCO_3^- was needed during the KE treatment compared with PL. In this way, as compared with the PL treatment, KE + $NaHCO_3$ co-ingestion significantly decreased HCO_3^- after warm-up and after the first 8-min and 30-s TT. Additionally, KE produced greater anion gap values than the PL after the warm-up and after the TTs carried out at the start of the testing session. Therefore, ketoacidosis and the greater concentration of HCO_3^- may be used in the buffering activity during the TT in the KE treatment than in the PL one, decreasing the reserve of extracellular HCO_3^-, and negatively affecting the export of molecules of H^+ and lactate from muscle cells (Hollidge-Horvat et al., 2000; Spriet et al., 1986). Despite the potential acid-base disturbances associated with ketone supplementation during exercise, the current study demonstrated an improvement in endurance performance. This suggests that the ergogenic effect of acute KE + $NaHCO_3$ appears to outweigh the negative impact of acid-base disturbances caused by ketones.

Table 3 Rating of Perceived Exertion and Gut Discomfort Results During the Study on Ketones or Placebo Ingestion

Outcome	Treatment	Baseline	Pre-WU	Post-WU	Post 8′TT	Post 30″TT	After 4.5-hr road cycling	Post second 8′TT	Post second 30″TT	Time × Treatment		
										F	*p*	η^2
RPE (a.u.)	Ketones	N/A	N/A	2.14 ± 1.65	7.57 ± 2.63	8.18 ± 2.48	3.75 ± 1.73	8.86 ± 1.33	9.46 ± 1.26	0.540	.804	.020
	Placebo			2.11 ± 1.59	7.39 ± 1.99	8.43 ± 2.08	4.04 ± 2.33	8.71 ± 1.82	9.29 ± 1.94			
Gut discomfort (a.u.)	Ketones	0.78 ± 1.30	1.17 ± 1.94	1.35 ± 1.87	1.44 ± 3.87	1.69 ± 3.01	1.77 ± 2.02	1.20 ± 1.90	1.16 ± 1.78	0.588	.765	.021
	Placebo	0.44 ± 0.81	1.05 ± 1.50	1.71 ± 2.69	1.78 ± 2.53	1.25 ± 2.52	1.21 ± 1.77	0.83 ± 1.40	0.60 ± 1.72			

Note. RPE = rating of perceived exertion; a.u. = arbitrary units; WU = warm-up; TT = time trial.

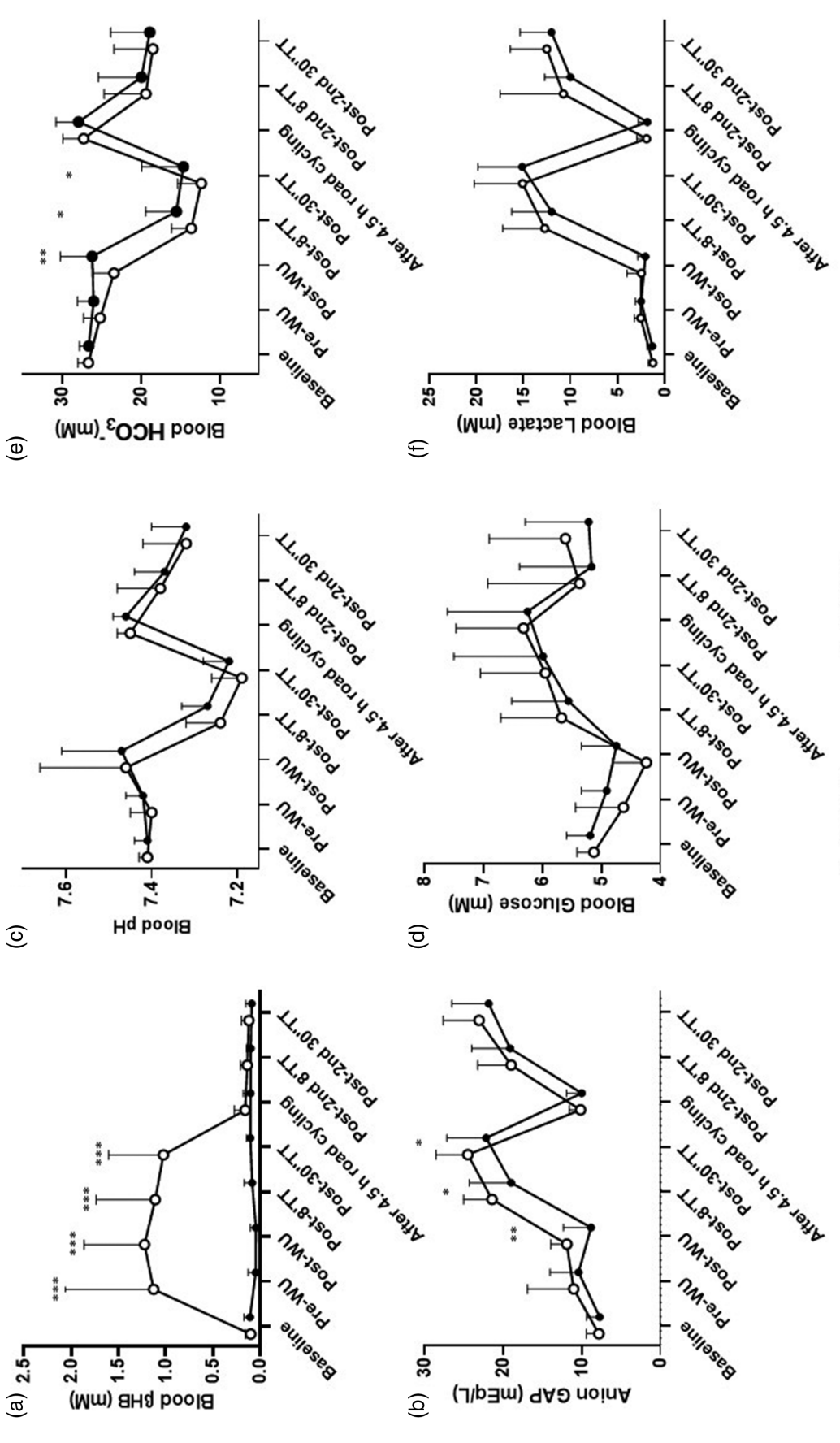

Figure 5 — Acid–base and metabolites results in both groups during the study. (a) βHB blood concentrations, (b) anion gap, (c) blood pH, (d) blood glucose, (e) blood bicarbonate, and (f) blood lactate concentrations. *Significant differences between KE and PL, $p \leq .05$; **$p \leq .01$; ***$p \leq .001$. WU = warm-up; TT = time trial; KE = ketone monoester; PL = placebo; βHB = β-hydroxybutyrate.

Notably, early research showed a linear relationship between ketone dose ingestion and gut discomfort and gastrointestinal symptoms (Stubbs et al., 2019), which can also affect performance in endurance-based tests. However, the cyclists in the present study presented very low values of gut discomfort after KE ingestion, with these results being similar those in the PL treatment. This finding is in accordance with previous studies (Evans et al., 2019; Poffé et al., 2020; Poffé, Wyns, et al., 2021) that reported a low incidence of gastrointestinal distress associated with low blood ketone concentrations.

From a practical perspective, although the current evidence supporting its application for improving cycling performance is limited, the results of the present study suggest that the acute ingestion of KE + $NaHCO_3$ could serve as an ergogenic aid for WorldTour cyclists. Particularly, this supplementation approach shows promise in road stages that last around 8 min, such as prologues. Moreover, it may also be beneficial in specific scenarios where cyclists need to initiate a breakaway at the start of a stage. Conversely, the main limitation of the present study was that gas analysis during exercise was not performed to obtain more results on metabolic outcomes. Additionally, the decrease in circulating ketones to baseline values by the end of the 4.5 hr cycling bout and for the second 8-min and 30-s TT, where it was unclear how long participants achieved hyperketonemia due to the impossibility to obtain a blood sample during the 4.5 hr of outdoor cycling was also a potential limitation of the study. However, the practical application of the results obtained from real field conditions, and the study sample of difficult-to-access athletes, would be considered as strengths of the present study.

Conclusions

This study demonstrated that acute supplementation of KE + $NaHCO_3$ improved cycling performance during an 8-min TT in WorldTour cyclists, which was conducted at the early stage of a stage simulation. Additionally, this supplementation led to the concurrent occurrence of hyperketonemia. However, no significant improvements in cycling performance were observed in the first 30-s TT, the 4.5 hr of outdoor cycling, or the TTs (8 min and 30 s) conducted later in the stage simulation among the WorldTour cyclists when utilizing the same acute supplementation of KE + $NaHCO_3$ co-ingestion.

Acknowledgments

The authors would like to thank all participants who gave their time and effort for completion of this research. This work received no other financial support and has no relationship to industry. The authors have no conflicts of interest to declare. **Author Contributions:** Ávila, López-Román, and Van Elslande were responsible for study conceptualization and experimental design. All authors were involved in the acquisition, research, interpretation, and analysis of data. Ramos-Campo wrote the manuscript. All authors critically revised the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

References

Ávila-Gandía, V., Torregrosa-García, A., Pérez-Piñero, S., Ortolano, R., Abellán-Ruiz, M.S., & López-Román, F.J. (2021). One-week high-dose β-alanine loading improves world tour cyclists' time-trial performance. *Nutrients, 13*(8), Article 543. https://doi.org/10.3390/nu13082543

Brooks, E., Lamothe, G., Nagpal, T.S., Imbeault, P., Adamo, K., Kara, J., & Doucet, É. (2022). Acute ingestion of ketone monoesters and precursors do not enhance endurance exercise performance: A systematic review and meta-analysis. *International Journal of Sport Nutrition and Exercise Metabolism, 32*(3), 214–225. https://doi.org/10.1123/ijsnem.2021-0280

Burke, L.M., & Hawley, J.A. (2018). Swifter, higher, stronger: What's on the menu? *Science, 362*(6416), 781–787. https://doi.org/10.1126/science.aau2093

Burke, L.M., & Maughan, R.J. (2015). The Governor has a sweet tooth—Mouth sensing of nutrients to enhance sports performance. *European Journal of Sport Science, 15*(1), 29–40. https://doi.org/10.1080/17461391.2014.971880

Carr, A.J., Hopkins, W.G., & Gore, C.J. (2011). Effects of acute alkalosis and acidosis on performance. *Sports Medicine, 41*(10), 801–814. https://doi.org/10.2165/11591440-000000000-00000

Cox, P.J., Kirk, T., Ashmore, T., Willerton, K., Evans, R., Smith, A., Murray, A.J., Stubbs, B., West, J., McLure, S.W., King, M.T., Dodd, M.S., Holloway, C., Neubauer, S., Drawer, S., Veech, R.L., Griffin, J.L., & Clarke, K. (2016). Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. *Cell Metabolism, 24*(2), 256–268. https://doi.org/10.1016/j.cmet.2016.07.010

Dearlove, D.J., Faull, O.K., Rolls, E., Clarke, K., & Cox, P.J. (2019). Nutritional ketoacidosis during incremental exercise in healthy athletes. *Frontiers in Physiology, 10,* Article 290. https://doi.org/10.3389/fphys.2019.00290

Dearlove, D.J., Harrison, O.K., Hodson, L., Jefferson, A., Clarke, K., & Cox, P.J. (2021). The effect of blood ketone concentration and exercise intensity on exogenous ketone oxidation rates in athletes. *Medicine & Science in Sports & Exercise, 53*(3), Article 505. https://doi.org/10.1249/MSS.0000000000002502

Egan, B., & D'Agostino, D.P. (2016). Fueling performance: Ketones enter the mix. *Cell Metabolism, 24*(3), 373–375. https://doi.org/10.1016/j.cmet.2016.08.021

Evans, M., Cogan, K.E., & Egan, B. (2017). Metabolism of ketone bodies during exercise and training: Physiological basis for exogenous supplementation. *Journal of Physiology, 595*(9), 2857–2871. https://doi.org/10.1113/JP273185

Evans, M., & Egan, B. (2018). Intermittent running and cognitive performance after ketone ester ingestion. *Medicine & Science in Sports & Exercise, 50*(11), 2330–2338. https://doi.org/10.1249/MSS.0000000000001700

Evans, M., McClure, T.S., Koutnik, A.P., & Egan, B. (2022). Exogenous ketone supplements in athletic contexts: Past, present, and future. *Sports Medicine, 52*(Suppl. 1), 25–67. https://doi.org/10.1007/s40279-022-01756-2

Evans, M., McSwiney, F.T., Brady, A.J., & Egan, B. (2019). No benefit of ingestion of a ketone monoester supplement on 10-km running performance. *Medicine & Science in Sports & Exercise, 51*(12), 2506–2515. https://doi.org/10.1249/MSS.0000000000002065

Grgic, J., Pedisic, Z., Saunders, B., Artioli, G.G., Schoenfeld, B.J., McKenna, M.J., Bishop, D.J., Kreider, R.B., Stout, J.R., Kalman, D.S., Arent, S.M., VanDusseldorp, T.A., Lopez, H.L., Ziegenfuss, T.N., Burke, L.M., Antonio, J., & Campbell, B.I. (2021). International Society of Sports Nutrition position stand: Sodium bicarbonate and exercise performance. *Journal of the International Society of Sports Nutrition, 18*(1), Article 61. https://doi.org/10.1186/s12970-021-00458-w

Harvey, K.L., Holcomb, L.E., & Kolwicz, S.C. (2019). Ketogenic diets and exercise performance. *Nutrients, 10*(11), Article 2296. https://doi.org/10.3390/nu11102296

Hollidge-Horvat, M.G., Parolin, M.L., Wong, D., Jones, N.L., & Heigenhauser, G.J.F. (2000). Effect of induced metabolic alkalosis on human skeletal muscle metabolism during exercise. *American Journal of Physiology—Endocrinology and Metabolism, 278*(2), E316–E329. https://doi.org/10.1152/ajpendo.2000.278.2.e316

Kerksick, C.M., Arent, S., Schoenfeld, B.J., Stout, J.R., Campbell, B., Wilborn, C.D., Taylor, L., Kalman, D., Smith-Ryan, A.E., Kreider, R.B., Willoughby, D., Arciero, P.J., VanDusseldorp, T.A., Ormsbee, M.J., Wildman, R., Greenwood, M., Ziegenfuss, T.N., Aragon, A.A., & Antonio, J. (2017). International society of sports nutrition position stand: Nutrient timing. *Journal of the International Society of Sports Nutrition, 14*(1), Article 33. https://doi.org/10.1186/s12970-017-0189-4

Leckey, J.J., Ross, M.L., Quod, M., Hawley, J.A., & Burke, L.M. (2017). Ketone diester ingestion impairs time-trial performance in professional cyclists. *Frontiers in Physiology, 8,* Article 806. https://doi.org/10.3389/fphys.2017.00806

Maizels, E.Z., Ruderman, N.B., Goodman, M.N., & Lau, D. (1977). Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat. *Biochemical Journal, 162*(3), 557–568. https://doi.org/10.1042/bj1620557

Margolis, L.M., & O'Fallon, K.S. (2020). Utility of ketone supplementation to enhance physical performance: A systematic review. *Advances in Nutrition, 11*(2), 412–419. https://doi.org/10.1093/advances/nmz104

McCarthy, D.G., Bone, J., Fong, M., Pinckaers, P.J.M., Bostad, W., Richards, D.L., van Loon, L.J.C., & Gibala, M.J. (2023). Acute ketone monoester supplementation impairs 20-min time-trial performance in trained cyclists: A randomized, crossover trial. *International Journal of Sport Nutrition and Exercise Metabolism, 33*(4), 181–188. https://doi.org/10.1123/ijsnem.2022-0255

Ohmori, H., Kawai, K., & Yamashita, K. (1990). Enhanced ketone body uptake by perfused skeletal muscle in trained rats. *Endocrinologia Japonica, 37*(3), 421–429. https://doi.org/10.1507/endocrj1954.37.421

O'Malley, T., Myette-Cote, E., Durrer, C., & Little, J.P. (2017). Nutritional ketone salts increase fat oxidation but impair high-intensity exercise performance in healthy adult males. *Applied Physiology, Nutrition and Metabolism, 42*(10), Article 641. https://doi.org/10.1139/apnm-2016-0641

Poffé, C., Ramaekers, M., Bogaerts, S., & Hespel, P. (2020). Exogenous ketosis impacts neither performance nor muscle glycogen breakdown in prolonged endurance exercise. *Journal of Applied Physiology, 128*(6), 1643–1653. https://doi.org/10.1152/japplphysiol.00092.2020

Poffé, C., Ramaekers, M., Bogaerts, S., & Hespel, P. (2021). Bicarbonate unlocks the ergogenic action of ketone monoester intake in endurance exercise. *Medicine & Science in Sports & Exercise, 53*(2), Article 431. https://doi.org/10.1249/MSS.0000000000002467

Poffé, C., Wyns, F., Ramaekers, M., & Hespel, P. (2021). Exogenous ketosis impairs 30-min time-trial performance independent of bicarbonate supplementation. *Medicine & Science in Sports & Exercise, 53*(5), 1068–1078. https://doi.org/10.1249/MSS.0000000000002552

Rhea, M.R. (2004). Determining the magnitude of treatment effects in strength training research through the use of the effect size. *The Journal of Strength & Conditioning Research, 18*(4), Article 40. https://journals.lww.com/nsca-jscr/Fulltext/2004/11000/DETERMINING_THE_MAGNITUDE_OF_TREATMENT_EFFECTS_IN.40.aspx

Robinson, A.M., & Williamson, D.H. (1980). Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiological Reviews, 60*(1), 143–187. https://doi.org/10.1152/physrev.1980.60.1.143

Spriet, L.L., Lindinger, M.I., Heigenhauser, G.J.F., & Jones, N.L. (1986). Effects of alkalosis on skeletal muscle metabolism and performance during exercise. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology, 251*(5), R833–R839. https://doi.org/10.1152/ajpregu.1986.251.5.r833

Stubbs, B.J., Cox, P.J., Kirk, T., Evans, R.D., & Clarke, K. (2019). Gastrointestinal effects of exogenous ketone drinks are infrequent, mild, and vary according to ketone compound and dose. *International Journal of Sport Nutrition and Exercise Metabolism, 29*(6), 596–603. https://doi.org/10.1123/ijsnem.2019-0014

Valenzuela, P.L., Morales, J.S., Castillo-García, A., & Lucia, A. (2020). Acute ketone supplementation and exercise performance: A systematic review and meta-analysis of randomized controlled trials. *International Journal of Sports Physiology and Performance, 15*(3), 298–308. https://doi.org/10.1123/ijspp.2019-0918

Winder, W.W., Holloszy, J.O., & Baldwin, K.M. (1974). Enzymes involved in ketone utilization in different types of muscle: Adaptation to exercise. *European Journal of Biochemistry, 47*(3), 461–467. https://doi.org/10.1111/j.1432-1033.1974.tb03713.x