Exogenous Ketosis Impairs 30-min Time-Trial Performance Independent of Bicarbonate Supplementation

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ABSTRACT

POFFÉ, C., F. WYNS, M. RAMAEKERS, and P. HESPEL. Exogenous Ketosis Impairs 30-min Time-Trial Performance Independent of Bicarbonate Supplementation. *Med. Sci. Sports Exerc.*, Vol. 53, No. 5, pp. 1068–1078, 2021. **Purpose:** We recently demonstrated that coingestion of $NaHCO_3$ to counteract ketoacidosis resulting from oral ketone ester (KE) intake improves mean power output during a 15-min time trial (TT) at the end of a 3-h cycling race by ~5%. This ergogenic effect occurred at a time when blood ketone levels were low, as ketosis was only induced during the initial ~2 h of the race. Therefore, in the current study, we investigated whether performance also increases if blood ketone levels are increased in the absence of ketoacidosis during high-intensity exercise. **Methods:** In a double-blind crossover design, 14 well-trained male cyclists completed a 30-min TT ($TT_{30'}$) followed by an all-out sprint at 175% of lactate threshold (SPRINT). Subjects were randomized to receive (i) 50 g KE, (ii) 180 mg·kg^{-1} body weight $NaHCO_3$ (BIC), (iii) KE + BIC, or (iv) a control drink (CON). **Results:** KE ingestion increased blood D-ß-hydroxybutyrate to ~3–4 mM during the $TT_{30'}$ and SPRINT ($P < 0.001$ vs CON). In KE, blood pH and bicarbonate concomitantly dropped, causing 0.05 units lower pH and 2.6 mM lower bicarbonate in KE compared with CON during the $TT_{30'}$ and SPRINT ($P < 0.001$ vs CON). BIC coingestion resulted in 0.9 mM higher blood D-ß-hydroxybutyrate ($P < 0.001$ vs KE) and completely counteracted ketoacidosis during exercise ($P > 0.05$ vs CON). Mean power output during $TT_{30'}$ was similar between CON and BIC at 281 W, but was 1.5% lower in the KE conditions (main effect of KE: $P = 0.03$). Time to exhaustion in the SPRINT was ~64 s in CON and KE and increased by ~8% in the BIC conditions (main effect of BIC: $P < 0.01$). **Discussion:** Neutralization of acid–base disturbance by BIC coingestion is insufficient to counteract the slightly negative effect of KE intake during high-intensity exercise. **Key Words:** KETONE, BICARBONATE, EXERCISE PERFORMANCE, KETOACIDOSIS

Over recent years, there has been keen interest in supplementation with oral ketone products as a potent nutritional intervention to enhance performance in endurance exercise. This is primarily due to a seminal study showing that acute ingestion of the ketone ester (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (KE) slightly increased distance covered during a 30-min time trial (TT) after 1-h submaximal cycling (1). In addition, we also reported that consistent ingestion of KE after

exercise counteracts the development of overreaching symptoms as well as improved endurance exercise performance during a 3-wk training overload period (2).

Originally, the ergogenic potential of exogenous ketosis was primarily attributed to the ability of the ketone bodies β-hydroxybutyrate (βHB) and acetoacetate to provide an alternative metabolic fuel for skeletal muscle, thereby sparing precious carbohydrate stores (3). Indeed, Cox et al. (1) showed that coingestion of ~40 g KE and ~70 g carbohydrates, compared with carbohydrate alone (~120 g), significantly inhibited the depletion of muscle glycogen stores during a 2-h submaximal (70% maximal oxygen uptake ($\dot{V}O_{2max}$)) cycling bout in the fasted state. Such action could be ergogenic in the final stage of exercise events in which glycogen availability is an important determinant of performance. However, during high-intensity efforts requiring a high glycolytic rate for optimal performance, glycolytic inhibition conceivably is ergolytic (4,5). Furthermore, although exogenous ketosis due to the intake of KE was positioned as the cause of the glycogen-sparing action reported by Cox et al., an alternative explanation is the ~40% lower carbohydrate intake before and during exercise in KE compared with the control condition, which *per se* reduces muscle glycogenolysis (6–8). In support of such hypothesis, we recently

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observed a ~3-h simulated cycling race in conjunction with ample carbohydrate intake before (~2 $g \cdot kg^{-1}$) and during (60 $g \cdot h^{-1}$) the exercise, to induce similar glycogen breakdown in the absence or presence of exogenous ketosis (9). In addition, high-intensity exercise performance at the end of the race was unaffected by the KE ingestion (9,10), which corroborates observations by others during a maximal incremental cycling (11), a 10-km all-out run after a 1-h submaximal running (12), and a ~15-min all-out cycling TT preceded by a 30-min submaximal cycling (13).

Another factor that may affect performance during exogenous ketosis is dysregulation of acid–base balance. We (9,10) and others (11) recently demonstrated that KE intake during exercise causes a mild acidosis (ketoacidosis), which in turn partly drains alkaline reserve because of blood HCO_3^- utilization. Although the causative role of extracellular H^+ accumulation in the development of muscular fatigue remains controversial (14), there is clear evidence that such extracellular acidosis is ergolytic (15). Hence, we recently hypothesized that a potential ergogenic effect of KE may be overruled by ketosis-induced acid–base dysregulation. Indeed, we recently demonstrated that counteracting KE-induced acidosis by concomitant oral bicarbonate ($NaHCO_3$) ingestion increased mean power output during a 15-min TT at the end of a ~3-h simulated cycling race. In contrast, neither $NaHCO_3$ nor KE alone affects performance (10), indicating a synergistic action of KE and $NaHCO_3$. Interestingly, this ergogenic effect occurred at a time when blood ketone levels had returned to baseline (~0.5 mM) after KE intake aimed to induce supposed glycogen sparing (~2–3 mM) during the initial 2 h of the race simulation. These observations clearly indicate that the synergistic action of KE + bicarbonate to stimulate high-intensity exercise performance at the end of a prolonged endurance exercise event occurred independent of a significant contribution of ketone bodies in energy metabolism because circulating ketone levels were very low during the performance test. Whether $NaHCO_3$ intake to negate ketoacidosis may also contribute to improve performance in a high-intensity exercise associated with high blood ketone levels during the exercise is currently unknown. Therefore, the present study was undertaken to investigate whether coingestion of KE and $NaHCO_3$ may enhance performance in a 30-min TT. A number of features were built into the study protocol to create high ecological validity with real-world cycling TTs. These involved the inclusion of a warm-up (WU) and the provision of adequate carbohydrate and fluid to mimic recommended practices of intake.

METHODS

Ethical approval and subjects. Highly-trained, male cyclists ($n = 12$; mean ± SD: age, 26 ± 6 yr; height, 1.79 ± 0.06 m; body mass, 70 ± 7 kg; $\dot{V}O_{2max}$, 62.5 ± 5.5 $mL \cdot kg^{-1} \cdot min^{-1}$ (range, 55–71 $mL \cdot kg^{-1} \cdot min^{-1}$); cycling activity, 8.9 ± 3.1 $h \cdot wk^{-1}$ (range, 6–15 $h \cdot wk^{-1}$)) conceivably consuming a carbohydrate-rich diet were recruited to participate in this study. All participants were nonsmokers, were not taking any medication or

ergogenic supplement during the last 3 months before the start of the study, and were instructed to maintain their habitual exercise training regimen and diet throughout the full study period. Subjects were fully informed of the content and potential risks involved with the experimental procedures before giving their written informed consent. Ethical approval (B322201939080) was obtained from the KU Leuven Biomedical Ethics Committee and conforms to the Declaration of Helsinki.

General study design. This double-blind, placebo-controlled study with a crossover design involved four experimental sessions, each separated by a 7-d washout period. The experimental sessions were conducted at the same time of the day and involved a 60-min standardized WU, followed by a 30-min cycling TT ($TT_{30'}$) and an all-out cycling bout at 175% of the lactate threshold (SPRINT; Fig. 1). Participants were randomized to receive the following four conditions in a stratified, randomized order (stratum: average power output during the $TT_{30'}$ in the second familiarization session): (i) KE, (ii) $NaHCO_3$ (BIC), (iii) KE + BIC, or (iv) placebo (CON). In the KE conditions (KE, KE + BIC), the subjects received in total 50 g (726 ± 75 $mg \cdot kg^{-1}$) of the KE (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (TdeltaS Ltd, Thame Oxfordshire, United Kingdom) to be ingested in two equal aliquots at (i) minutes 30 and (ii) 55 of WU. This dosing strategy was based on pilot testing showing a stable physiological ketosis (3–4 mM βHB) throughout the $TT_{30'}$ and SPRINT. The other conditions (BIC, CON) received a taste, volume, and viscosity-matched placebo containing 12.5% w/v collagen peptan® (6d Sports Nutrition, Oudenaarde, Belgium) and 1 mM bitter sucrose octaacetate (Sigma-Aldrich, Bornem, Belgium) dissolved in water. To avoid potential visual identification of the treatments, both drinks were provided in nontransparent 50-mL tubes. In the BIC conditions (BIC, KE + BIC), the subjects received a total of 180 $mg \cdot kg^{-1}$ body weight $NaHCO_3$. They received 90 $mg \cdot kg^{-1}$ with breakfast 2 h before the start of WU, followed by another 30 $mg \cdot kg^{-1}$ 1 h before WU, both in the form of 700 mg gelatin capsules. Furthermore, throughout the WU, the subjects received two boluses of 30 $mg \cdot kg^{-1}$ dissolved in 250 mL of a 6% maltodextrin solution (15 g carbohydrates) to be ingested during the first and second half of WU. The other conditions (KE, CON) received an appearance-matched placebo (NaCl) containing an equimolar amount of sodium. Furthermore, at minute 30 of WU, the subjects ingested an energy gel (6d Sports Nutrition) delivering 30 g carbohydrates in order to obtain a carbohydrate intake of 60 $g \cdot h^{-1}$ in each experimental condition during the WU. All exercise tests were performed in an air-conditioned laboratory (18°C, 60% relative humidity) using each subject's own race bike mounted on a calibrated cycle ergometer (Avantronic Cyclus II, Leipzig, Germany).

Preliminary testing. Two weeks before the start of the study, the subjects completed two sessions to be familiarized with the experimental procedures. In the first session, each subject's $\dot{V}O_{2max}$ rate and lactate threshold (LT) were determined using the lactate minimum power concept (Dausin C. et al., 2020). Subjects first performed an incremental cycling test (100 W plus 40 W every 8 min) during which capillary
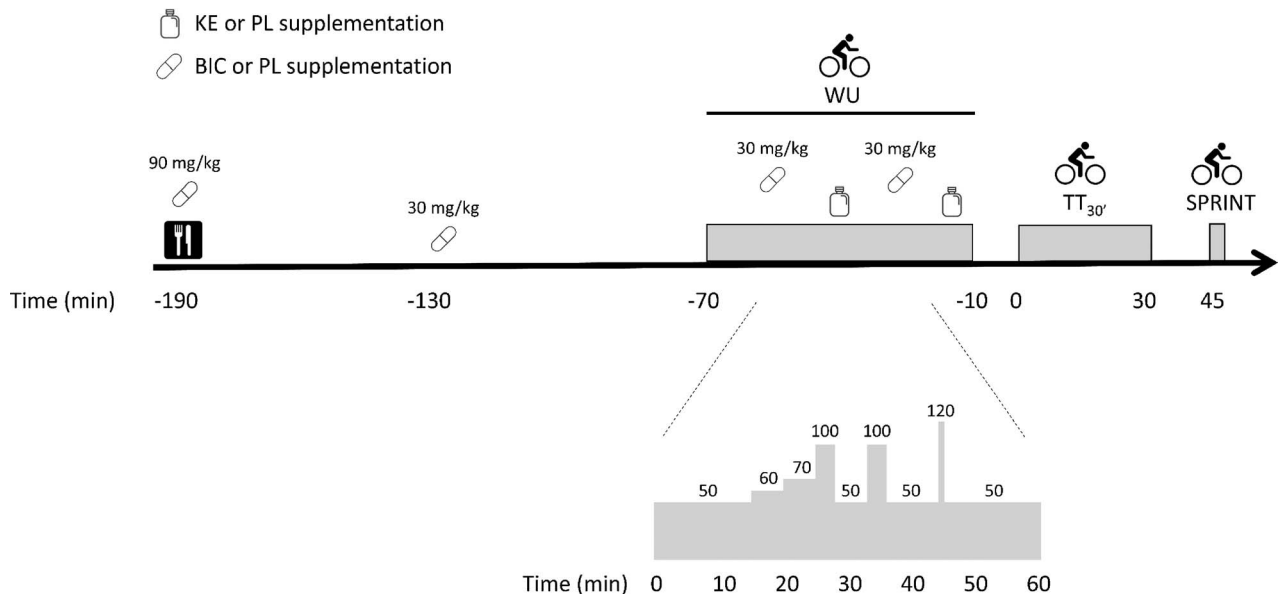
FIGURE 1—Schematic representation of the experimental sessions. The study involved four experimental sessions in a double-blind, randomized, crossover design. At each occasion, subjects ($n = 12$) performed a 60-min WU during which they cycled at low to moderate exercise intensities (50%–70% of LT) alternated by three high-intensity efforts (2×3 min at 100% of LT and 1×1 min at 120% of LT) while gradually ingesting 60 g carbohydrates. After WU, subjects performed a 30-min simulated cycling TT ($TT_{30'}$) and a ~1-min all-out cycling bout at 175% of the LT (SPRINT). Before and during the exercise protocol, subjects received placebo (CON), ketone ester (KE), bicarbonate (BIC), or ketone ester plus bicarbonate (KE + BIC). Intensities during WU were set relative to each individual's LT.

blood samples were obtained at minutes 4 and 8 of each intensity block for determination of blood lactate levels (Lactate Pro2; Arkray, Kyoto, Japan). LT was immediately determined as the lowest workload corresponding to a 1-mM blood lactate increment from minutes 4 to 8 within the same stage. After reaching LT, the subjects recovered during 5 min (cycling at 100 W) before starting a ramp test (100 W plus 25 W every 30 s) to exhaustion for determination of $\dot{V}O_{2max}$. Respiratory gas exchange was measured continuously during the ramp test (Cortex Metalyzer II, Leipzig, Germany), and the highest oxygen uptake measured over a 10-s period was defined as the $\dot{V}O_{2max}$. After 10 min of active recovery by cycling at 100 W, the subjects performed the $TT_{30'}$ and SPRINT (see discussion hereinafter). On the second visit, the subjects completed the full exercise protocol (WU–$TT_{30'}$–SPRINT) as to be performed during the later experimental trials.

Experimental sessions. The evening before each experimental session, the subjects received a standardized carbohydrate-rich dinner (~5600 kJ (82 ± 9 kJ·kg^{-1}): 69% carbohydrate (3.37 ± 0.35 g·kg^{-1}), 16% fat (0.35 ± 0.04 g·kg^{-1}), and 15% protein (0.75 ± 0.08 g·kg^{-1})). Next morning, they reported to the laboratory between 6:00 AM. and 10:00 AM. and received a standardized breakfast (~2600 kJ (38 ± 4 kJ·kg^{-1}): 72% carbohydrate (1.62 ± 0.17 g·kg^{-1}), 13% fat (0.13 ± 0.01 g·kg^{-1}), and 15% protein (0.34 ± 0.04 g·kg^{-1})) followed by 500 mL of a 6% carbohydrate drink (6d Sports Nutrition) 60 min later. Two hours after the ingestion of breakfast, the subjects started the exercise protocol. The protocol started with a 60-min WU during which the subjects cycled at low to moderate intensities (50%–70% of LT) alternated by three high-intensity efforts (2×3 min at 100% of LT and 1×1 min at 120% of LT).

Upon completion of the WU followed by a 10-min rest period, the subjects started the $TT_{30'}$ in which they aimed for the highest possible mean power output. Workload during the first 5 min (t0–t5) was set at the mean power output of the $TT_{30'}$ during the second familiarization session. From t5 to t25, the subjects could voluntarily adjust the workload every 5 min according to their perception of fatigue. From t25 to t30, adjustments were allowed each minute to establish full exhaustion by the end of the $TT_{30'}$. After completion of the $TT_{30'}$, the subjects passively recovered for 10 min followed by 5-min cycling at 50 W. Finally, they performed a constant-load (175% of LT) exercise bout (SPRINT) to exhaustion (cadence ≤70 rpm). Water was provided *ad libitum* during the first experimental session, and intake was recorded to allow for identical intake during the next sessions. Heart rate was continuously measured during the full exercise protocol but was blinded to the subjects. A countdown timer together with actual cadence was shown during WU and $TT_{30'}$, whereas only cadence was shown during the SPRINT. Standardized verbal encouragement was only given during the SPRINT.

Assessment of perceived exertion, appetite, and gastrointestinal discomfort. RPE values (6–20 Borg scale) were assessed immediately after completion of the WU, $TT_{30'}$, and SPRINT. Perceptions of appetite and gastrointestinal (GI) discomfort were rated 6 min after completion of the SPRINT by means of a validated 10-point visual analog scale (16) and a 0–8 Likert scale questionnaire (17), respectively. Appetite scores were assessed using four questions: "How hungry do you feel?" "How full do you feel?" "How satisfied do you feel?" "How much do you think you could eat now?" GI distress was quantified at the systemic (dizziness, headache, muscle cramp, urge to

urinate), upper (reflux, bloating, nausea, vomiting), and lower abdominal (cramps, flatulence, abdominal pain, diarrhea) level.

Capillary and venous blood sampling and analyses. Blood D-βHB and glucose levels were immediately determined (Glucomen Lx plus-meter with Lx ketone and Lx glucose strips) from capillary blood samples obtained from a hyperemic earlobe at the start and halfway of the WU and at the start, mid, and end of the $TT_{30'}$. An investigator who was otherwise not involved in the experiments performed both measurements to ensure double blindness. Blood lactate concentrations were measured (Lactate Pro 2; Arkray) in a capillary blood sample from the earlobe before exercise and at regular intervals during the $TT_{30'}$ as well as 5 min after completion of the SPRINT. In addition, a 70-μL capillary blood sample was collected from a preheated earlobe into a safeCLINITUBE (Radiometer Medical APS, Copenhagen, Denmark) (i) before breakfast, at the start of the (ii) WU and (iii) $TT_{30'}$, and after completion of the (iv) $TT_{30'}$ and (v) SPRINT. After hypoxic mixing for 10 s, samples were analyzed using an automated acid–base laboratory (ABL90 Flex analyzer; Radiometer Medical Aps, Copenhagen, Denmark) for acid–base balance, blood gasses, and electrolytes. Furthermore, venous blood samples from an antecubital vein (Venoject, Terumo, Tokyo, Japan) were collected into vacuum tubes containing EDTA (Becton Dickinson (BD Vacutainer)) immediately before and after the $TT_{30'}$. Samples were immediately centrifuged (1500 rpm for 15 min at 4°C) to separate plasma, which was stored at −80°C until assayed by a colorimetric reagent kit (Wako Chemicals, Neuss, Germany) for nonesterified free fatty acid (FFA) concentration.

Urine sampling and analyses. Subjects were required to empty their bladder upon arrival at the laboratory after which all urine was collected in flasks up to the end of SPRINT. Urinary output was noted and urinary ketone excretion was evaluated using ketone reagent strips (Ketostix; Ascensia Diabetes Care). Fluid intake was standardized from the evening before until the end of each experimental session.

Statistical analyses. Statistical analyses were performed in GraphPad Prism version 8.4.2 (La Jolla, CA). Differences between conditions over time were evaluated using a three-way (time–KE–BIC) repeated-measures ANOVA, whereas alterations between conditions at one time point were assessed using a two-way (KE–BIC) repeated-measures ANOVA. If the sphericity assumption was violated (Mauchly's test), a Geisser–Greenhouse correction was applied. In case of a significant interaction effect, *post hoc* analyses were included using Bonferroni correction and reported P values refer to these *post hoc* analyses. Otherwise, P values for main effects were included. Outlier statistics were performed using the ROUT method (maximum desired false discovery rate, 1%). One outlier was detected and removed for time to exhaustion in the SPRINT. Statistical significance was defined as $P < 0.05$. All data are presented as mean ± SD, together with effect sizes (ES). ES values were presented as partial η squared (η_p^2) for main and interaction effects, and if appropriate, as Cohen's d for *post hoc* pairwise comparisons. Cohen's d ES values were interpreted using thresholds of <0.25, >0.25, ≥0.5, and ≥1.0 for

trivial, small, medium, and large, respectively (18). Ninety-five percent confidence intervals (CI) were included for the performance outcomes. An *a priori* power analysis in G*Power (version 3.1) indicated that a sample size of eight participants was required to detect a significant interaction effect ($P < 0.05$) for mean power output during the $TT_{30'}$ (primary outcome) in a two-way repeated-measures ANOVA. Input parameters ($\eta_p^2 = 0.44$; statistical power = 0.80; correlation among repeated measures = 0.68) were calculated based on our earlier study showing an ergogenic effect of KE + BIC during the $TT_{30'}$ at the end of a simulated cycling race (10). Anticipating a 1:3 dropout in a worst-case scenario, we included 12 participants in the study.

RESULTS

Blood βHB concentration and urinary ketone excretion. A time–KE–BIC interaction effect was detected for blood βHB levels ($P = 0.02$, $\eta_p^2 = 0.23$; Fig. 2A). Baseline blood βHB levels were similar at ~0.2 mM between the conditions ($P > 0.05$) and remained stable in CON and BIC. Conversely, blood βHB increased after KE intake and was stable at ~3.5 mM throughout the $TT_{30'}$ in KE ($P < 0.001$ vs CON at the start, mid, and end of the $TT_{30'}$). Coingestion of BIC resulted in a further increase in blood [βHB] (+0.9 mM compared with KE alone, $d = 1.12$, $P < 0.001$ at the start, mid, and end of the $TT_{30'}$), causing a stable ketosis at ~4.5 mM during the $TT_{30'}$ in KE + BIC. No urinary ketone bodies were detected during CON or BIC, whereas small amounts were excreted in KE (0.03 ± 0.02 g) and KE + BIC (0.05 ± 0.03 g; main effect of KE, $P < 0.001$). Urine volume was not affected by any of the experimental conditions as indicated by the absence of a main (KE, $P = 0.37$; BIC, $P = 0.67$) or interaction ($P = 0.32$) effect.

Blood glucose, lactate, and plasma FFA concentrations. Initial blood glucose and lactate levels were similar between conditions (Figs. 2B, C). There was a time–KE effect for blood glucose ($P < 0.001$, $\eta_p^2 = 0.57$), indicating that KE and KE + BIC lowered blood glucose levels by ~0.6 mM at the start ($P < 0.001$, $d = 1.91$) and at the end ($P < 0.001$, $d = 0.67$) of the $TT_{30'}$ compared with CON and BIC. For blood lactate, time–KE ($\eta_p^2 = 0.57$) and time–BIC ($\eta_p^2 = 0.44$) interaction effects (both, $P < 0.001$) were found. At the start of $TT_{30'}$, blood lactate levels were similar at ~1.5 mM between conditions ($P > 0.05$). In CON, $TT_{30'}$ and SPRINT increased blood lactate levels up to ~7 and ~10.5 mM, respectively (both, $P < 0.001$ vs baseline). KE suppressed blood lactate concentrations at the end of the $TT_{30'}$ (~5 mM, $d = 0.59$) and SPRINT (~8.5 mM, $d = 0.72$), whereas BIC resulted in higher lactate levels ($TT_{30'}$: ~9.5 mM, $d = 0.61$; SPRINT: ~12.5 mM, $d = 0.53$; all, $P < 0.001$ vs CON). Conversely, lactate levels in KE + BIC were similar to CON both at the end of the $TT_{30'}$ and SPRINT. For plasma FFA concentrations, a time–KE effect was present ($P < 0.01$, $\eta_p^2 = 0.68$). Plasma FFA levels at the start of the $TT_{30'}$ were similar between KE and KE + BIC compared with CON and BIC at ~0.08 mM ($P = 0.15$). $TT_{30'}$ increased plasma FFA concentrations in
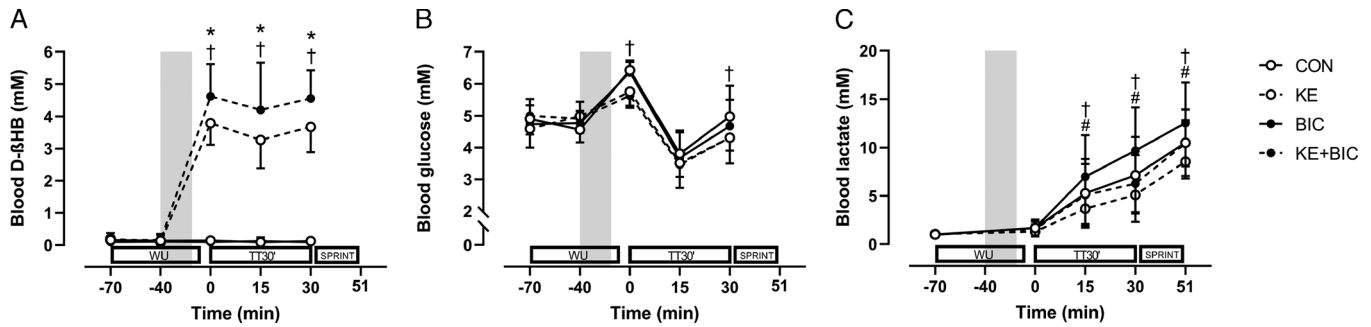
FIGURE 2—Effect of KE intake, alone or combined with bicarbonate supplementation on blood D-βHB, glucose, and lactate during the exercise protocol. Data are mean ± SD ($n = 12$) for blood D-βHB (A), glucose (B), and lactate (C) concentrations. Subjects performed a 60-min WU followed by a 30-min simulated TT ($TT_{30'}$) and a ~1-min SPRINT while receiving either control (-○-, CON), ketone ester (-○·, KE), bicarbonate (-●-, BIC), or ketone ester plus bicarbonate supplements (-●·, KE + BIC). Gray area depicts time zone during which KE supplements were administered. †$P < 0.05$ for effect of KE (KE and KE + BIC vs CON and BIC); #$P < 0.05$ for effect of BIC (BIC and KE + BIC vs CON and KE); *$P < 0.05$ for KE + BIC vs KE.

CON and BIC ($P < 0.001$ vs pre-$TT_{30'}$) but not in KE and KE + BIC ($P = 0.30$ vs pre-$TT_{30'}$). Hence, upon completion of the $TT_{30'}$, plasma [FFA] was about twofold higher in CON and BIC (both, 0.16 ± 0.08) than in KE (0.08 ± 0.04) and KE + BIC (0.09 ± 0.03, KE vs no KE: $P < 0.001$, $d = 1.32$).

Acid–base balance and arterial pCO₂. Both time–KE ($\eta_p^2 = 0.86$ and 0.90 for blood pH and [HCO₃⁻], respectively) and time–BIC ($\eta_p^2 = 0.79$ and 0.85 for blood pH and [HCO₃⁻], respectively) interaction effects were found for blood pH and [HCO₃⁻] (all, $P < 0.001$; Figs. 3A, B). Baseline blood pH (~7.40; range, 7.37–7.45) and [HCO₃⁻] (25.8 mM; range, 24.0–28.6 mM) were similar between conditions ($P > 0.05$). However, ingestion of KE during the WU resulted in ~0.05 units lower blood pH ($d = 3.69$) and ~3.4 mM lower blood HCO₃⁻ concentration ($d = 3.66$) by the start of the $TT_{30'}$ compared with CON ($P < 0.001$). Conversely, BIC ingestion caused blood pH and [HCO₃⁻] to increase, respectively, by ~0.07 units ($d = 4.41$) and by ~4.6 mM ($d = 5.62$) above CON values ($P < 0.001$). Consequently, blood pH and [HCO₃⁻] were similar in KE + BIC compared with CON by the start of the $TT_{30'}$. $TT_{30'}$ decreased blood pH to a similar extent (ΔpH, −0.09) in all conditions. However, for drop in blood [HCO₃⁻], a main

effect of both BIC ($P < 0.01$, $\eta_p^2 = 0.64$) and KE ($P = 0.01$, $\eta_p^2 = 0.70$) was observed. Thus, compared with CON (−5.9 ± 3.1 mM), during the $TT_{30'}$, blood [HCO₃⁻] dropped more in BIC (−7.9 ± 3.1 mM, $d = 0.64$) versus less in KE (−4.6 ± 1.6 mM, $d = 0.52$). Conversely, blood [HCO₃⁻] drop during the $TT_{30'}$ (−5.7 ± 2.4) was similar between KE + BIC and CON. SPRINT decreased blood pH to a larger extent in KE and KE + BIC (−0.08), compared with CON and BIC (−0.06; main effect of KE: $P = 0.02$, $d = 0.52$). Conversely, drop in [HCO₃⁻] during the SPRINT was similar (~2.8 mM) between all conditions. Nonetheless, because of the differences existing at the start of the $TT_{30'}$, at the end of the $TT_{30'}$, and the SPRINT, blood pH and [HCO₃⁻] were ~0.06 units and ~2.2 mM lower in KE than in CON (all, $P < 0.001$). Conversely, blood pH and [HCO₃⁻] after the $TT_{30'}$ and SPRINT, respectively, were not significantly different between KE + BIC and CON. For pCO₂, a time–KE interaction effect was detected ($P < 0.001$, $\eta_p^2 = 0.40$), indicating that KE ingestion during WU caused a slightly lower pCO₂ in KE and KE + BIC (~40.0 mm Hg) than in CON or BIC (~41.8 mm Hg) at the start of the $TT_{30'}$ ($P < 0.001$, $d = 0.87$), but not at the other time points (all, $P > 0.05$).
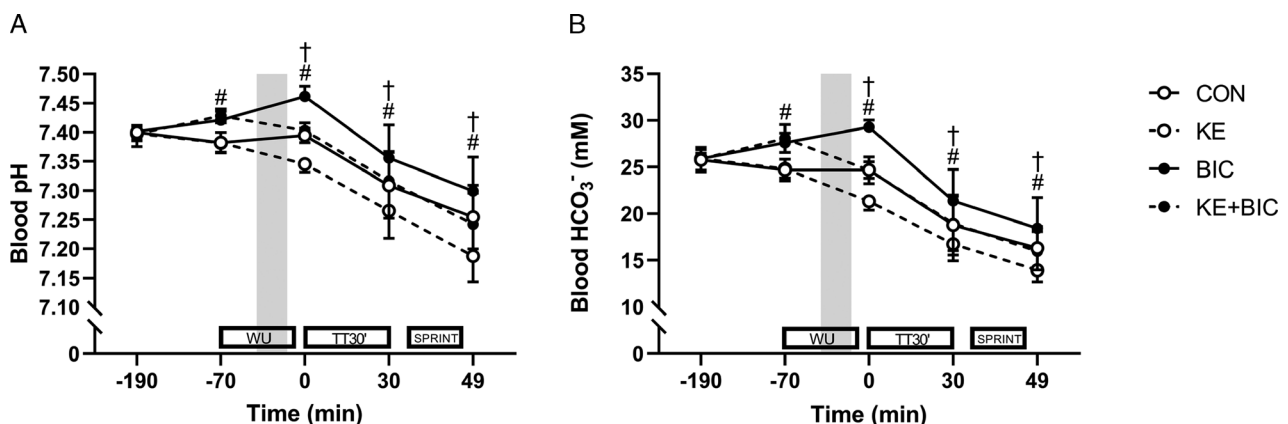

FIGURE 3—Effect of KE and/or bicarbonate supplementation on blood pH and bicarbonate concentration. Data are mean ± SD and represent blood pH (A) and bicarbonate (HCO₃⁻) concentration (B). In a crossover design, subjects ($n = 12$) received control (-○-, CON), ketone ester (-○·, KE), bicarbonate (-●-, BIC), or ketone ester and bicarbonate (-●·, KE + BIC). Gray area depicts time zone during which subjects received the KE supplement. †$P < 0.05$ for effect of KE (KE and KE + BIC vs CON and BIC); #$P < 0.05$ for effect of BIC (BIC and KE + BIC vs CON and KE).

Exercise performance. There was a main effect of KE ($P = 0.03$, $\eta_p^2 = 0.33$) for mean power output during the $TT_{30'}$, indicating that mean power output during the $TT_{30'}$, independent of BIC, on average was 3.8 ± 1.5 W (95% CI, -7.2 to -0.5 W, $d = 0.13$) lower in KE and KE + BIC (~277 W) than in CON and BIC (~281 W; Figs. 4A–C). In addition, KE and KE + BIC performances taken together (mean values for KE and KE + BIC), power output decreased in 8 of 12 subjects, whereas it was stable or slightly increased in 4. Performance drop in KE and KE + BIC was also positively correlated with the actual increase in blood βHB affected by the KE intake ($r = 0.51$, $P = 0.01$). For time to exhaustion in the SPRINT, there was a main effect of BIC ($P < 0.01$, $\eta_p^2 = 0.39$), indicating that time to exhaustion was 5 ± 1 s (95% CI, $+2$ to $+8$ s, $d = 0.30$) longer in BIC and KE + BIC (~69 s) compared with CON and KE (~64 s; Figs. 4D–F). No order effect was present for both these performance outcomes ($P = 0.36$ and $P = 0.60$ for $TT_{30'}$ and SPRINT, respectively).

Plasma electrolytes. For plasma calcium levels, time–KE ($\eta_p^2 = 0.38$) and time–BIC ($\eta_p^2 = 0.49$) effects were found (both, $P < 0.001$). KE intake during the WU resulted in ~0.02 mM higher plasma calcium levels during the $TT_{30'}$ and SPRINT compared with CON (all, $P < 0.01$). Conversely, BIC ingestion induced ~0.03 mM lower plasma calcium levels than in CON (all, $P < 0.001$). Hence, plasma calcium levels were similar between CON and KE + BIC at all times ($P > 0.05$). For plasma sodium concentrations, a time–KE effect was

observed ($P = 0.02$, $\eta_p^2 = 0.24$), indicating that plasma [Na$^+$] was ~1 mM higher in KE and KE + BIC compared with CON and BIC, at the start and end of both the $TT_{30'}$ and SPRINT (all, $P < 0.05$). For plasma chloride levels, both time–KE ($P = 0.01$, $\eta_p^2 = 0.34$) and time–BIC ($P < 0.001$, $\eta_p^2 = 0.96$) effects were detected. KE intake marginally ($+0.5$ mM) increased plasma chloride levels at the end of the $TT_{30'}$ and SPRINT (both, $P < 0.05$), whereas BIC ingestion substantially (-5.5 mM) lowered plasma [Cl$^-$] at the start and end of the $TT_{30'}$ and after the SPRINT (all, $P < 0.001$). No differences between the conditions were observed for plasma potassium concentrations (Table 1).

RPE and heart rate. For RPE, a time–KE interaction effect was detected ($P = 0.02$, $\eta_p^2 = 0.30$). *Post hoc* analyses indicated that RPE was similar between all experimental conditions during the WU and $TT_{30'}$, but tended to be lower in KE and KE + BIC (18.4 ± 1.1) than in CON and BIC (18.9 ± 0.9) during the SPRINT ($P = 0.07$, $d = 0.49$). Heart rate during the $TT_{30'}$ and peak heart rate after the SPRINT were similar between all conditions as evidenced by the absence of main or interaction effects (Table 2).

GI distress and appetite perception. The overall incidence and severity of GI symptoms were low. Nonetheless, there was a main effect of KE for GI distress ($P = 0.01$, $\eta_p^2 = 0.51$), indicating that GI distress was slightly higher in KE (12 ± 12 on a 96-point scale) and KE + BIC (11 ± 9) compared with CON (7 ± 12) and BIC (5 ± 8). This resulted from increased distress at the systemic (dizziness) and upper
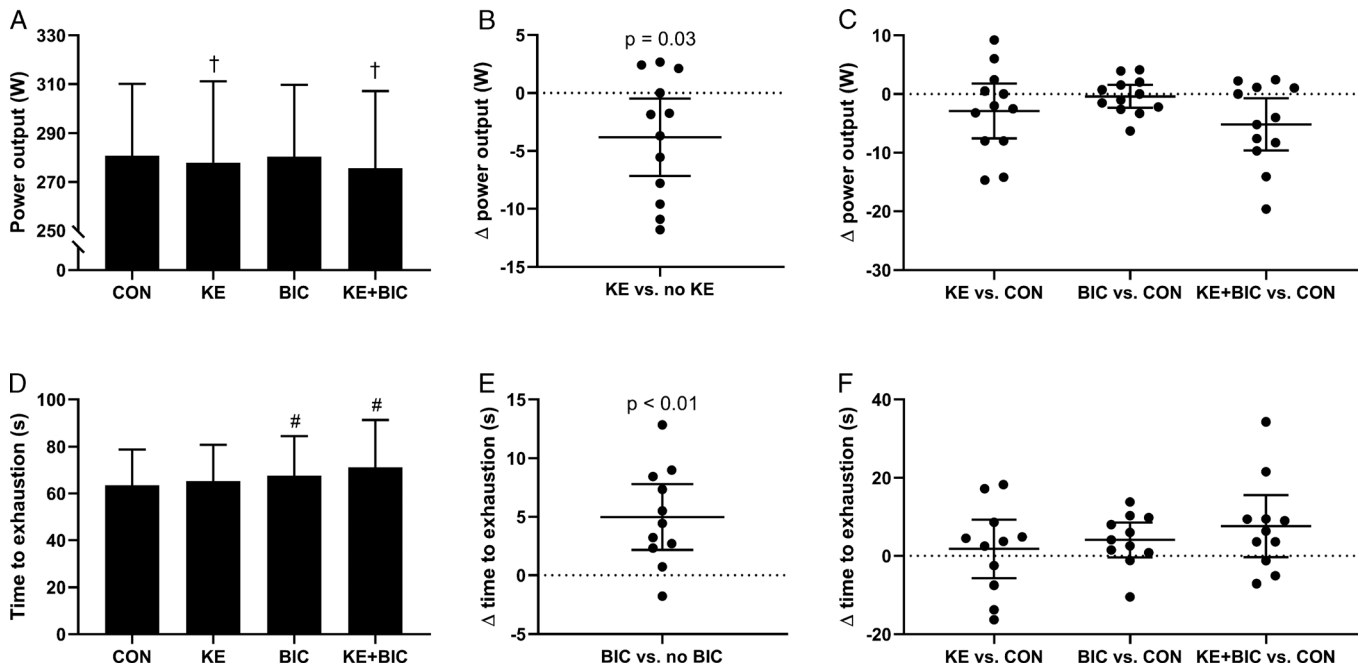

FIGURE 4—Effect of KE and/or bicarbonate supplementation on exercise performance. Data are mean ± SD and represent average power output during the 30-min simulated TT ($TT_{30'}$) (A) and individual differences between KE and KE + BIC vs CON and BIC together with mean ± 95% CI (B), as well as individual differences between each experimental condition compared with CON with mean ± 95% CI (C). Mean ± SD for time to exhaustion in the SPRINT (D) and individual differences between BIC and KE + BIC vs CON and KE together with mean ± 95% CI (E), as well as individual differences between each experimental condition compared with CON with mean ± 95% CI (F). Subjects ($n = 12$) received in a crossover design control (CON), ketone ester (KE), bicarbonate (BIC), or ketone ester and bicarbonate (KE + BIC) before and during exercise. †$P < 0.05$ for effect of KE (KE and KE + BIC vs CON and BIC); #$P < 0.05$ for effect of BIC (BIC and KE + BIC vs CON and KE).

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TABLE 1. Effect of KE and/or bicarbonate supplementation on plasma electrolytes.

	−190 min Breakfast	−70 min Start WU	0 min Start $TT_{30'}$	30 min End $TT_{30'}$	49 min End SPRINT
Plasma [Ca^{2+}]					
CON	1.18 ± 0.05	1.19 ± 0.04	1.17 ± 0.04	1.19 ± 0.04	1.20 ± 0.04
KE	1.19 ± 0.04	1.19 ± 0.03	1.20 ± 0.05*	1.22 ± 0.04*	1.23 ± 0.03*
BIC	1.19 ± 0.04	1.18 ± 0.06	1.14 ± 0.06**	1.15 ± 0.05**	1.16 ± 0.05**
KE + BIC	1.19 ± 0.04	1.17 ± 0.02	1.16 ± 0.03*,**	1.18 ± 0.03*,**	1.19 ± 0.04*,**
Plasma [Na^+]					
CON	140.6 ± 1.8	143.1 ± 1.8	143.0 ± 1.0	145.3 ± 1.2	145.5 ± 1.5
KE	140.9 ± 1.7	142.6 ± 1.0	143.8 ± 1.0*	146.7 ± 1.7*	146.7 ± 1.8*
BIC	140.7 ± 1.7	141.9 ± 1.9	142.7 ± 1.1	145.3 ± 1.4	145.1 ± 1.3
KE + BIC	141.1 ± 1.7	142.5 ± 1.0	143.5 ± 1.6*	146.5 ± 1.5*	146.6 ± 1.6*
Plasma [Cl^-]					
CON	105.6 ± 1.5	108.6 ± 1.3	108.7 ± 1.4	110.3 ± 1.4	109.7 ± 1.5
KE	105.2 ± 1.5	108.3 ± 1.6	108.5 ± 1.2	110.8 ± 1.8*	109.9 ± 1.4*
BIC	105.4 ± 1.3	104.5 ± 0.9**	103.0 ± 1.2**	104.5 ± 1.3**	103.8 ± 1.2**
KE + BIC	105.3 ± 1.9	104.5 ± 1.6**	103.3 ± 1.7**	105.1 ± 1.7*,**	104.6 ± 1.7*,**
Plasma [K^+]					
CON	6.3 ± 1.4	5.7 ± 0.8	5.2 ± 0.4	6.1 ± 0.6	4.9 ± 0.4
KE	6.2 ± 0.9	5.8 ± 0.8	5.2 ± 0.8	5.8 ± 0.6	4.6 ± 0.5
BIC	6.0 ± 1.2	5.9 ± 1.1	5.0 ± 0.5	5.7 ± 0.9	4.6 ± 0.4
KE + BIC	6.1 ± 1.0	5.5 ± 0.5	5.1 ± 0.6	5.6 ± 0.7	4.4 ± 0.4

Values are mean ± SD in mM for plasma calcium (Ca^{2+}), sodium (Na^+), chloride (Cl^-), and potassium (K^+) concentration before breakfast (−190 min), warming up (−70 min), start (0 min) and end (30 min) of $TT_{30'}$, and end of SPRINT (49 min). In a crossover design, subjects ($n = 12$) received control (CON), ketone (KE), bicarbonate (BIC), or ketone and bicarbonate (KE + BIC). BIC supplementation was started at −190 min, whereas KE supplementation was started at −40 min during the warming up.
*$P < 0.05$ for effect of KE (KE and KE + BIC vs CON and BIC).
**$P < 0.05$ for effect of BIC (BIC and KE + BIC vs CON and KE).

abdominal (bloating, nausea, vomiting) levels in KE and KE + BIC (all, $P < 0.05$). Scores on each of the four "appetite questions" were similar between the experimental conditions.

Identification of condition and best performance trial. Upon completion of the final experimental session, the subjects were asked to identify the sequential order of their experimental conditions and their best $TT_{30'}$ performance. None of the participants correctly identified the four experimental conditions, whereas 9 of 12 subjects correctly identified their best $TT_{30'}$ performance.

DISCUSSION

It has been previously suggested that elevated blood βHB concentration due to KE intake inhibits glycolytic flux during exercise (1). However, such effect did not emerge during prolonged (~3 h) submaximal exercise in conjunction with ample carbohydrate intake (9), but still might occur during shorter (<60 min) and higher-intensity endurance exercise bouts requiring no carbohydrate intake to stimulate performance via a metabolic role (19). Given the pivotal role of muscle glycogen breakdown in energy turnover in such exercise context, ketosis-induced glycolytic inhibition obviously may be detrimental to performance. Furthermore, ketoacidosis associated with KE ingestion (9,20) is a potential additional ergolytic factor to be considered whenever attempting to use exogenous ketosis as an ergogenic nutritional strategy. Interestingly in this regard, we recently demonstrated that counteracting ketoacidosis by concomitant ingestion of $NaHCO_3$ elevated mean power output by ~5% during a 15-min TT at the end of a 3-h simulated cycling race (10). However, this effect late in the event occurred at a time when circulating blood βHB concentration had reverted to <0.5 mM after a 3- to 5-mM peak earlier in the exercise. This excludes the theory that βHB could have significantly

affected energy turnover or performance during the TT either by serving as an energy substrate or by inhibition of glycolysis. Therefore, in the present study, we investigated the effect of coingestion of $NaHCO_3$ with KE, aiming to acutely raise blood βHB level to 3–5 mM in the absence of ketoacidosis, on physiological responses and performance in a 30-min TT. In order to optimize the ecological validity of the trial, the protocol included warming up and nutritional procedures that are typically recommended in the context of elite cycling TT performance. Exogenous ketosis was induced by KE intake during warming up, which expectedly elevated blood βHB to ~3–5 mM during the TT. In line with our earlier findings (10), $NaHCO_3$ coingestion was fully effective in preventing acidosis related to oral ketone ingestion. However, in contrast to our hypothesis, independent of whether $NaHCO_3$ was coingested, exogenous ketosis slightly impaired TT performance. The current data taken together with literature data (12,13,21) clearly indicate that acute KE ingestion must be dissuaded in the context of short maximal endurance exercise events lasting shorter than 1 h.

Previous studies have yielded equivocal results with regard to the effects of exogenous ketosis on exercise performance

TABLE 2. Effect of KE and/or bicarbonate supplementation on RPE and heart rate.

	CON	KE	BIC	KE + BIC
RPE (6–20)				
WU	10.8 ± 1.0	11.0 ± 1.0	10.3 ± 1.0	10.8 ± 1.3
$TT_{30'}$	18.1 ± 0.8	17.4 ± 1.6	17.3 ± 0.9	17.4 ± 1.2
SPRINT	18.8 ± 1.1	18.4 ± 1.1*	19.0 ± 0.6	18.4 ± 1.2*
HR, bpm				
$TT_{30'}$	167 ± 14	168 ± 11	168 ± 14	168 ± 13
SPRINT	177 ± 14	176 ± 12	178 ± 13	179 ± 11

Values are mean ± SD for RPE at the end of WU, $TT_{30'}$, and SPRINT. HR is represented as mean ± SD for average HR during $TT_{30'}$ and peak HR during SPRINT. In a crossover design, subjects ($n = 12$) received control (CON), ketone (KE), bicarbonate (BIC) or ketone and bicarbonate (KE + BIC) before and during exercise.
*$P = 0.07$ for effect of KE (KE and KE + BIC vs CON and BIC).

(1,9,11,12,21–27). The ongoing controversy is at least partly due to the use of different sources of exogenous ketones. In contrast to ketone salts (22,24,27), ketone body precursors (25,26), and ketone diesters (21), only the ketone monoester (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (KE) has been demonstrated to be able to consistently raise blood βHB levels greater than 1 mM during exercise (1,9–11). As a result, in contrast to other ketone supplements, the intake of ketone monoester intake is often associated with a small and transient reduction in blood pH (20). In a seminal study, KE was shown to enhance performance by 2% in a 30-min TT preceded by a 1-h constant-load cycling bout at 75% of W_{max} (1). However, it is likely that the performance benefits of KE in this study are probably mainly, if not entirely, explained by the specific conditions of the protocol: a 90-min exercise bout undertaken after an overnight fast and with differences between the treatment and placebo trials in terms of total carbohydrate intake. This design includes the confounding effect of differences in carbohydrate intake and a lack of real-world context. However, in later studies using recommended rates of carbohydrate provision before and during exercise, KE failed to improve performance during a 10-km all-out run (12), a ~15-min cycling TT (13), and a 15-min TT at the end of a 3-h 15-min simulated cycling race (9,10). Accordingly, in the conditions of the current study, KE intake alone improved performance neither in the $TT_{30'}$ nor in the SPRINT. However, and as clearly shown in Figure 4C, individual responses to bicarbonate ingestion alone (BIC) were different from responses to KE alone or in combination with BIC. Subjects clearly exhibited a "zero response" to oral bicarbonate alone, with individual delta power outputs between BIC and CON in the $TT_{30'}$ being <1.5%, which in fact indicates excellent reproducibility of the performance measurements. In contrast, irrespective of the addition of BIC, during KE, mean power output produced in the $TT_{30'}$ in most subjects was either lower or equal to CON, with no difference between KE and KE + BIC. Because $TT_{30'}$ power outputs clearly were inert to BIC, we also evaluated individual performances independent of BIC (mean value for KE and KE + BIC). This analysis demonstrated that KE decreased power output during the $TT_{30'}$ on average by 1.5% (95% CI, −0.2% to −2.6%). It should be emphasized that, although this effect was considered trivial ($d = 0.13$) by statistical analysis, it might still be highly relevant in the context of elite athletic performance. Additional support for such conclusion comes from the observation that the degree of performance decrement during the $TT_{30'}$ in KE and KE + BIC correlated with the extent of blood βHB elevation by KE. This may also explain why a previous study found no effect of KE establishing low blood βHB (~1.5 mM) on performance in a ~40-min endurance event (12). Conversely, in the current protocol, the subjects worked at 3–5 mM circulating blood βHB concentration. It is also important to note that we cannot exclude that GI symptoms also contributed to the performance outputs measured. The incidence and severity of GI symptoms were very low, indeed, yet nonetheless slightly higher in the KE versus no KE conditions. In contrast, GI distress was absent in previous studies showing no effect on performance at lower blood ketone levels (9,10,12). Such assumption is in line with earlier observations by Stubbs and coworkers (28) showing a dose-dependent increase in GI discomfort after KE ingestion at rest.

High-dose KE ingestion may also affect performance by disturbing acid–base balance. Previous studies have demonstrated that KE intake transiently decreases blood pH and buffering capacity by reducing blood HCO_3^- concentration, both at rest (20) and during exercise (9–11). Accordingly, in the conditions of the current study, KE ingestion during the warm-up decreased blood pH and bicarbonate level from the start to the end of the exercise protocol. Nonetheless, these acid–base shifts due to KE did not seem to affect performance because power outputs in the $TT_{30'}$ were independent of $NaHCO_3$ coingestion. At first glance, this finding is in contrast with our recent report showing that coingestion of KE and $NaHCO_3$ increased 15-min TT performance at the end of a 3-h 15-min simulated cycling race (10). However, in the latter study, blood ketone levels were only elevated during the initial 2 h of submaximal exercise from a ~3-h race simulation, and as such, the ergogenic effect occurred at a time when blood ketone levels had returned to ~0.5 mM at normal blood pH. In contrast, blood ketone levels here were greater than 3 mM during the performance measurements in the $TT_{30'}$ and SPRINT.

The current and earlier findings (1,9,12,13) taken together do not allow us to identify the precise physiological mechanisms by which exogenous ketosis differentially affects performance in various exercise contexts. However, clearly the presence of high blood βHB during exercise is not a prerequisite for oral ketone administration to exert an ergogenic effect (10). In contrast, high blood βHB is more likely to become ergolytic in all-out exercise bouts involving a high fraction of anaerobic ATP production due to premature absorption of extracellular buffering capacity (HCO_3^-), preexercise pH drop, higher incidence of GI symptoms, and regulation of lactate metabolism.

Concerning the latter mechanism, equivocal results have been reported in recent literature. Cox et al. (1) first reported KE intake to suppress blood lactate accumulation during exercise by inhibition of muscle glycogen breakdown. However, as indicated before, the direct effect of KE on energy substrate metabolism in this study was confounded by markedly higher rate of carbohydrate intake before and during exercise in the control condition compared with KE. We recently reported that, compared with a control condition in which an identical rate of carbohydrate intake was provided (60 g·h^{-1}), KE failed to affect the exercise-induced increase in blood lactate during 3 h of intermittent submaximal exercise at 60%–90% of the LT (9). This is in line with other observations that KE does not alter changes in blood lactate concentration during moderate exercise intensities (11,12). However, discordant findings have been reported with regard to blood lactate accumulation during maximal exercise events. Acute exogenous ketosis due to KE intake suppressed blood lactate accumulation during the final stages of an incremental cycling test (11), but not during a ~40-min all-out run (12). However, blood βHB concentration in the latter

study may have been too low to regulate lactate metabolism. In the conditions of the current study, KE markedly suppressed blood lactate accumulation during the $TT_{30'}$ but not during the SPRINT. In fact the effect of KE to suppress blood lactate increase during the $TT_{30'}$ largely occurred during the initial half of the trial, where after further increments during the latter half, were similar between KE and CON. This may indicate that, at low circulating blood lactate concentrations, βHB inhibits lactate efflux from muscle cells via competitive inhibition of monocarboxylate transporters. However, as the exercise is continued, this inhibition is negated by increasing intramyocellular and extracellular lactate concentrations.

This hypothesis is supported by previous studies showing an inhibition of lactate transport by ketone bodies at low (~1 mM) but not at high (>6 mM) circulating lactate concentrations (29,30). Whether a lower rate of lactate production results from ketone-induced inhibition of glycolytic flux (31) or alterations in lactate trafficking (29) remains to be established. In this regard, it is also important to note that KE intake abolished the rise in plasma FFA concentrations during the $TT_{30'}$, which is likely to increase rather than decrease glycolytic flux (32,33). Furthermore, the contribution of FFA oxidation to total ATP production at workloads exceeding the maximal lactate steady-state during the $TT_{30'}$ conceivably was minor (6). It is probable that the lower plasma FFA concentrations during KE result largely, if not entirely, from βHB-induced suppression of adipolysis either via agonism of the nicotinic acid receptor PUMA-G (34) or via inhibition of catecholamine secretion (35).

We recently reported that BIC unlocked the ergogenic action of KE during a 15-min TT at the end of a 3-h simulated cycling race (10). Such ergogenic effect was completely absent in the current protocol. These divergent results may be at least partly explained by different blood pH and $[HCO_3^-]$ responses between these studies. In our earlier study, both blood pH and $[HCO_3^-]$ were higher in the KE + BIC compared with the control condition by the start of the 15-min TT after the 3-h race simulation. This resulted in substantially more HCO_3^- being used in buffering activity during the TT in KE + BIC than in CON (10). Conversely, in the conditions of the current study, blood pH and $[HCO_3^-]$ at the start of the $TT_{30'}$ were similar between KE + BIC and CON, which resulted in similar rates of HCO_3^- utilization during the $TT_{30'}$. Increased versus decreased extracellular HCO_3^- reserve stimulates versus impairs, respectively, the export of H^+ and La^- molecules from muscle cells (36–38). Hence, differential blood pH and HCO_3^- responses probably at least partly explain the opposite effects of KE and KE + BIC on blood lactate and exercise performance between our recent studies.

It has been postulated that there might be an optimal range for blood βHB concentration to improve performance (4,39). The suggested lower bound of the threshold ([βHB] >1 to 2 mM) is questioned by our earlier report showing a clear ergogenic effect of KE + BIC at ~0.5 mM blood βHB during a 15-min TT. Evans et al. (4) speculated that the upper bound of the optimal range may be at ~3 mM, with higher levels being deleterious because of increased side effects such as

ketoacidosis and GI distress. Our current findings support the latter rationale because higher blood [βHB] correlated with a greater degree of performance impairment. Along such rationale, in line with our earlier observations (10), coingestion of $NaHCO_3$ enhanced the blood βHB response to KE (Fig. 2A), which is probably due to elevated hepatic ketone body production (40). As a result, an overelevation in ketone levels may have downregulated performance outcomes slightly more in KE + BIC than in KE alone (Fig. 4C).

KE, alone or combined with $NaHCO_3$, slightly impaired performance in $TT_{30'}$. In contrast, time to exhaustion in SPRINT was 10% higher in KE + BIC than in KE or CON. This effect was clearly attributable to $NaHCO_3$ because BIC alone also improved performance in SPRINT. This finding is in line with numerous previous studies showing an ergogenic effect of $NaHCO_3$ during a short high-intensity exercise (for reviews, see Refs. [15,41,42]). This ergogenic effect is primarily attributed to increased buffering capacity (42,43). Nonetheless, performance also increased in KE + BIC despite similar blood $[HCO_3^-]$ levels to those of CON both before and after SPRINT. This indicates that other mechanisms are also likely implicated in the ergogenic effect of KE + BIC. Intriguingly, several studies have indicated that alterations in strong ion gradients between the intracellular, interstitial, and extracellular milieu and affecting sarcolemmal excitability and contractile function may also play a role in the ergogenic effect of $NaHCO_3$ (42,44). In this respect, we observed that $NaHCO_3$, both in the presence and absence of KE intake, slightly lowered plasma calcium and chloride levels before and after SPRINT. These alterations are in line with our recent study showing a decrease in plasma $[Ca^{2+}]$ and $[Cl^-]$ during a ~3-h simulated cycling race after $NaHCO_3$ intake independent of KE coingestion (10). However, the $NaHCO_3$-induced decrease in the concentration of both electrolytes was 50% smaller in the current compared with our previous study. Further research needs to elucidate the concerted actions of KE and $NaHCO_3$ on the intracellular and extracellular balance of strong ions and its potential consequences for exercise performance.

Such hypothesis that the effects of ketone bodies on exercise performance are rather dependent on their nonmetabolic functions is supported by two recent studies showing only minimal (<5%) contribution of ketone bodies to mitochondrial ATP production during exercise whenever carbohydrate availability is ample (45,46). As such, the contribution of ketone bodies to energy production in the current study was likely marginal. Nonetheless, a recent study showed that a 24-h incubation of human myotubes with 0.5 mM βHB, but not 1.5 or 5.0 mM, increased mitochondrial ATP production with more than 30%, although pyruvate was sufficiently available (47). This suggests that in conditions wherein ketone body availability is consistently elevated, such as following a long-term ketogenic diet, ketone bodies may still become a relevant energy substrate (48). Therefore, further research is required to evaluate whether the effects of KE on endurance exercise performance may be different in keto-adapted athletes.

In conclusion, this study shows that acute exogenous ketosis established by KE intake during warming up for a 30-min maximal exercise event slightly impairs performance independent of whether ketoacidosis is prevented by $NaHCO_3^-$ coingestion. Our current findings taken together with literature data clearly indicate that acute exogenous ketosis is not an optimal nutritional strategy in the context of short high-intensity endurance exercise performance.

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