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ABSTRACT

Purpose: We recently demonstrated that co-ingestion of NaHCO_3 to counteract ketoacidosis resulting from oral ketone ester (KE) intake improves mean power output during a 15-min TT at the end of a 3h 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 ~2h 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, fourteen well-trained male cyclists completed a 30-min time-trial ($\text{TT}_{30'}$) followed by an all-out sprint at 175% of lactate threshold (SPRINT). Subjects were randomized to receive either i) 50g ketone ester (KE), ii) 180 $\text{mg}\cdot\text{kg}^{-1}$ body weight NaHCO_3 (BIC), iii) KE+BIC or iv) a control drink (CON). **Results:** KE ingestion increased blood D- β -hydroxybutyrate to ~3-4 mM during $\text{TT}_{30'}$ 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 to CON during $\text{TT}_{30'}$ and SPRINT ($p<0.001$ vs. CON). BIC co-ingestion resulted in 0.9 mM higher blood D- β -hydroxybutyrate ($p<0.001$ vs. KE) and completely counteracted ketoacidosis during exercise ($p>0.05$ vs. CON). Mean power output during $\text{TT}_{30'}$ was similar between CON and BIC at 281W, but was 1.5% lower in the KE conditions (main effect of KE: $p=0.03$). Time-to-exhaustion in SPRINT was ~64s 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 co-ingestion is insufficient to counteract the slightly negative effect of KE intake during high-intensity exercise. **Keywords:** ketone, bicarbonate, exercise performance, ketoacidosis

INTRODUCTION

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 following 1h submaximal cycling (1). In addition, we also reported that consistent ingestion of KE post-exercise counteracts the development of overreaching symptoms as well as improved endurance exercise performance during a 3-week training overload period (2).

Originally the ergogenic potential of exogenous ketosis was primarily attributed to the ability of the ketone bodies β -hydroxybutyrate (BHB) and acetoacetate (AcAc) to provide an alternative metabolic fuel for skeletal muscle, thereby sparing precious carbohydrate stores (3). Indeed, Cox et al. (1) showed that co-ingestion of ~40g KE and ~70g carbohydrates, compared with carbohydrate alone (~120g), significantly inhibited the depletion of muscle glycogen stores during a 2h submaximal (70% $\dot{V}O_2$ max) 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 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 to the control condition, which per se reduces muscle glycogenolysis (6–8). In support of such hypothesis, we recently observed a ~3h simulated cycling race in conjunction with ample carbohydrate intake before (~2 g·kg⁻¹) and

during ($60 \text{ g}\cdot\text{h}^{-1}$) 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 ketone ester ingestion (9, 10), which corroborates observations by others during maximal incremental cycling (11), a 10km all-out run following 1h submaximal running (12) and a ~15 min all-out cycling time-trial preceded by 30 min submaximal cycling (13).

Another factor that may impact performance during exogenous ketosis is dysregulation of acid-base balance. We (9, 10) and others (11) recently demonstrated that ketone ester intake during exercise causes a mild acidosis (ketoacidosis), which in turn partly drains alkaline reserve due to blood HCO_3^- utilisation. Even though 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 time-trial at the end of a ~3h simulated cycling race. In contrast, neither NaHCO_3 nor KE alone impacted 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) following ketone ester intake aimed to induce supposed glycogen sparing (~2-3 mM) during the initial 2 hours 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 NaHCO₃ intake to negate ketoacidosis may also contribute to improve performance in high-intensity exercise associated with high blood ketone levels during the exercise is currently unknown. Therefore, the present study was undertaken to investigate whether co-ingestion of KE and NaHCO₃ may enhance performance in a 30-min time-trial. A number of features were built into the study protocol to create high ecological validity with real-world cycling time trials. These involved the inclusion of a warm-up 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, age: 26 ± 6 (mean $\pm$ SD) y; height: 1.79 ± 0.06 m; body mass: 70 ± 7 kg; $\dot{V}O_2$ max: 62.5 ± 5.5 ml·kg⁻¹·min⁻¹, range: 55 – 71 ml·kg⁻¹·min⁻¹; cycling activity: 8.9 ± 3.1 h·wk⁻¹, range: 6 – 15 h·wk⁻¹] conceivably consuming a carbohydrate-rich diet were recruited to participate in this study. All participants were non-smokers, not taking any medication or ergogenic supplement during the last three 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 crossover design involved four experimental sessions, each separated by a 7-day washout period. The experimental sessions were conducted at the same time of the day and involved a 60 min

standardized warm-up (WU), followed by a 30 min cycling time-trial (TT_{30'}) and an all-out cycling bout at 175% of the lactate threshold (SPRINT) (Figure 1). Participants were randomized to receive the following four conditions in a stratified, randomized order (stratum: average power output during TT_{30'} in the second familiarization session): (i) ketone ester (KE), (ii) NaHCO₃ (BIC), (iii) KE+BIC, or (iv) placebo (CON). In the KE conditions (KE, KE+BIC), subjects received in total 50g ($726 \pm 75 \text{ mg}\cdot\text{kg}^{-1}$) of the ketone ester (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (TdeltaS Ltd, Thame Oxfordshire, UK) to be ingested in two equal aliquots at (i) min 30 and (ii) 55 of WU. This dosing strategy was based on pilot testing showing a stable physiological ketosis (3-4 mM β Hb) throughout 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 non-transparent 50 ml tubes. In the BIC conditions (BIC, KE+BIC), subjects received a total of $180 \text{ mg}\cdot\text{kg}^{-1}$ body weight NaHCO₃. They received $90 \text{ mg}\cdot\text{kg}^{-1}$ with breakfast 2h before the start of WU, followed by another $30 \text{ mg}\cdot\text{kg}^{-1}$ one hour before WU, both in the form of 700 mg gelatin capsules. Furthermore, throughout WU subjects received two boluses of $30 \text{ mg}\cdot\text{kg}^{-1}$ dissolved in 250 ml of a 6% maltodextrin solution (15g 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 min 30 of WU subjects ingested an energy gel (6d Sports Nutrition, Oudenaarde, Belgium) delivering 30g carbohydrates in order to obtain a carbohydrate intake of $60 \text{ g}\cdot\text{h}^{-1}$ in each experimental condition during the WU. All exercise testing was 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 maximal oxygen uptake rate ($\dot{V}O_2$ max) and lactate threshold (LT) was determined using the lactate minimum power (LMP) concept (Dausin et al., submitted). Subjects first performed an incremental cycling test (100 W plus 40 W every 8 min) during which capillary blood samples were obtained at min 4 and 8 of each intensity block for determination of blood lactate levels (Lactate Pro2, Arkray, Japan). LT was immediately determined as the lowest workload corresponding to a 1 mM blood lactate increment from min 4 to 8 within the same stage. After reaching LT, 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 $\dot{V}O_2$ max. 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 $\dot{V}O_2$ max. After 10 min of active recovery by cycling at 100 W, the subjects performed the TT_{30'} and SPRINT (see below). 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), 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), 15% protein (0.34 ± 0.04 g·kg⁻¹)] followed by 500 mL of a 6% carbohydrate drink (6d Sports Nutrition, Oudenaarde, Belgium) 60 minutes later. Two hours following ingestion of breakfast, 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 3 high-intensity efforts (2x 3 min at 100% of LT and 1x 1 min at 120% of LT). Upon completion of WU followed by a 10 min rest period, subjects started TT_{30'} in which they aimed for the highest possible mean power output. Workload during the first five minutes (t0-t5) was set at the mean power output of TT_{30'} during the second familiarization session. From t5 to t25, 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 TT_{30'}. After completion of TT_{30'} subjects passively recovered for 10 minutes followed by 5 min cycling at 50W. Finally, they performed a constant-load (175% of LT) exercise bout (SPRINT) to exhaustion (cadence $\leq$ 70 rpm). Water was provided ad libitum during the first experimental session, and intake was recorded to allow 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'}, while only cadence was shown during SPRINT. Standardized verbal encouragement was only given during SPRINT.

Assessment of perceived exertion, appetite and gastrointestinal discomfort. Ratings of perceived exertion (RPE, 6-20 Borg Scale) were assessed immediately after completion of WU, TT_{30'} and SPRINT. Perceptions of appetite and gastrointestinal discomfort were rated 6 min after completion of SPRINT by means of a validated 10-points visual analogue scale (VAS) (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?”. Gastrointestinal 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, diarrhoea) level.

Capillary and venous blood sampling and analyses. Blood D- β -hydroxybutyrate (β HB) and glucose levels were immediately determined (Glucomen Lx plus-meter with Lx ketone and Lx glucose strips) from capillary blood samples obtained from a hyperaemic earlobe at the start and halfway of WU and at the start, mid and end of 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, Japan) in a capillary blood sample from the earlobe before exercise and at regular intervals during TT_{30'} as well as 5 minutes after completion of SPRINT. In addition, a 70 μ l capillary blood sample was collected from a preheated earlobe into a safeCLINITUBE (Radiometer Medical APS, Copenhagen, Denmark) (i) before breakfast, (ii) at the start of WU (iii) and TT_{30'}, (iv) and after completion of TT_{30'} (v) and SPRINT. Following hypoxic mixing during 10 s, samples were analysed using an automated acid-base laboratory (ABL90 Flex analyser, 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 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, USA). Differences between conditions over time were evaluated using a three-way (time x KE x BIC) repeated-measures analysis of variance, while alterations between conditions at one timepoint were assessed using a two-way (KE x BIC) repeated-measures analysis of variance. 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 $\pm$ SD, together with effect sizes (ES). ES were presented as partial eta-squared (η_p^2) for main and interaction effects and if appropriate as Cohen's d for post-hoc pair-wise comparisons. Cohen's d effect sizes were interpreted using thresholds of <0.25 , >0.25 ≥ 0.5 and ≥ 1.0 for trivial, small, medium and large, respectively (18). 95% 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 analysis of variance. 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 a TT_{30'} at the end of a simulated cycling race (10). Anticipating a 1:3 dropout in a worst case scenario, twelve participants were included in the study.

RESULTS

Blood β HB concentration and urinary ketone excretion. A time x KE x BIC interaction effect was detected for blood β HB levels ($p = 0.02$, $\eta_p^2 = 0.23$, Figure 2A). Baseline blood β HB levels were similar at ~0.2 mM between the conditions ($p > 0.05$), and remained stable in CON and BIC. Conversely, blood β HB increased following KE intake and was stable at ~3.5 mM throughout TT_{30'} in KE ($p < 0.001$ vs. CON at start, mid and end of TT_{30'}). Co-ingestion of BIC resulted in a further increase in blood [β HB] (+ 0.9 mM compared to KE alone, $d = 1.12$, $p < 0.001$ at start, mid and end of TT_{30'}), causing a stable ketosis at ~4.5 mM during 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 free fatty acid concentrations. Initial blood glucose and lactate levels were similar between conditions (Figure 2B and 2C). There was a time x KE effect for blood glucose ($p < 0.001$, $\eta_p^2 = 0.57$), indicating that KE as well as 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 TT_{30'} compared to CON and BIC. For blood lactate, a time x KE ($\eta_p^2 = 0.57$) and a time x BIC ($\eta_p^2 = 0.44$) interaction effect (both $p < 0.001$) was 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 mM and ~10.5 mM, respectively (both $p < 0.001$ vs. baseline). KE suppressed blood lactate concentrations at the end of TT_{30'} (~5 mM, $d = 0.59$) and SPRINT (~8.5 mM, $d = 0.72$), while 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 TT_{30'} and SPRINT. For plasma FFA concentrations a time x KE effect was present ($p < 0.01$, $\eta_p^2 = 0.68$). Plasma FFA levels at the start of TT_{30'} were similar between KE and KE+BIC compared to CON and BIC at ~0.08 mM ($p = 0.15$). TT_{30'} increased plasma FFA concentrations in 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 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 pCO_2 . Both a time x KE ($\eta_p^2 = 0.86$ and 0.90 for blood pH and $[HCO_3^-]$, respectively) and a time x BIC ($\eta_p^2 = 0.79$ and 0.85 for blood pH and $[HCO_3^-]$, respectively) interaction effect was found for blood pH and $[HCO_3^-]$ (all $p < 0.001$, Figure 3A and 3B). Baseline blood pH (~7.40, range: 7.37–7.45) and $[HCO_3^-]$ (25.8 mM, range: 24.0–28.6

mM) were similar between conditions ($p > 0.05$). However, ingestion of KE during WU resulted in ~ 0.05 units lower blood pH ($d = 3.69$) and ~ 3.4 mM lower blood HCO_3^- concentration ($d = 3.66$) by the start of $\text{TT}_{30'}$ compared to CON ($p < 0.001$). Conversely, BIC ingestion caused blood pH and $[\text{HCO}_3^-]$ 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 $[\text{HCO}_3^-]$ were similar in KE+BIC compared to CON by the start of $\text{TT}_{30'}$. $\text{TT}_{30'}$ decreased blood pH to a similar extent (ΔpH : -0.09) in all conditions. Yet, for drop in blood $[\text{HCO}_3^-]$ 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 to CON (-5.9 ± 3.1 mM), during $\text{TT}_{30'}$ blood $[\text{HCO}_3^-]$ 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 $[\text{HCO}_3^-]$ drop during $\text{TT}_{30'}$ (-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 to CON and BIC (-0.06 , main effect of KE: $p = 0.02$, $d = 0.52$). Conversely, drop in $[\text{HCO}_3^-]$ during SPRINT was similar (~ 2.8 mM) between all conditions. Nonetheless, due to the differences existing at the start of $\text{TT}_{30'}$, at the end of $\text{TT}_{30'}$ and SPRINT blood pH and $[\text{HCO}_3^-]$ were ~ 0.06 units and ~ 2.2 mM lower in KE than in CON (all $p < 0.001$). Conversely, blood pH and $[\text{HCO}_3^-]$ after $\text{TT}_{30'}$ and SPRINT, respectively, were not significantly different between KE+BIC and CON. For pCO_2 , a time $\times$ KE interaction effect was detected ($p < 0.001$, $\eta_p^2 = 0.40$) indicating that KE ingestion during WU caused slightly lower pCO_2 in KE and KE+BIC (~ 40.0 mmHg) than in CON or BIC (~ 41.8 mmHg) at the start of $\text{TT}_{30'}$ ($p < 0.001$, $d = 0.87$), but not at the other time points (all $p > 0.05$).

Exercise performance. There was a main effect of KE ($p = 0.03$, $\eta_p^2 = 0.33$) for mean power output during TT_{30'}, indicating that mean power output during 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, Figure 4A-C). In addition, KE and KE+BIC performances taken together (mean values for KE and KE+BIC), power output decreased in 8 out of 12 subjects whilst 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 β HB effected by the ketone ester intake ($r = 0.51$, $p = 0.01$). For time to exhaustion in 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 to CON and KE (~64 s, Figure 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 (Table 1). For plasma calcium levels a time x KE ($\eta_p^2 = 0.38$) as well as a time x BIC ($\eta_p^2 = 0.49$) effect was found (both $p < 0.001$). KE intake during WU resulted in ~0.02 mM higher plasma calcium levels during TT_{30'} and SPRINT compared to 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 x KE effect was observed ($p = 0.02$, $\eta_p^2 = 0.24$) indicating that plasma $[\text{Na}^+]$ was ~1 mM higher in KE and KE+BIC compared to CON and BIC, at the start and at the end of both TT_{30'} and SPRINT (all $p < 0.05$). For plasma chloride levels, both a time x KE ($p = 0.01$, $\eta_p^2 = 0.34$) and a time x BIC ($p < 0.001$, $\eta_p^2 = 0.96$) effect were detected. KE intake marginally (+0.5 mM) increased plasma chloride levels at the

end of TT_{30'} and SPRINT (both $p < 0.05$), while BIC ingestion substantially (-5.5 mM) lowered plasma [Cl⁻] at the start and end of TT_{30'} and following SPRINT (all $p < 0.001$). No differences between the conditions were observed for plasma potassium concentrations.

RPE and heart rate (Table 2). For rate of perceived exertion, a time x KE interaction effect was detected ($p = 0.02$, $\eta_p^2 = 0.30$). Post-hoc analyses indicated that rate of perceived exertion was similar between all experimental conditions during 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 TT_{30'} as well as peak heart rate following SPRINT were similar between all conditions as evidenced by the absence of main or interaction effects.

Gastrointestinal distress and appetite perception. The overall incidence and severity of GI symptoms was low. Nonetheless there was a main effect of KE for gastrointestinal distress ($p = 0.01$, $\eta_p^2 = 0.51$), indicating that gastrointestinal distress was slightly higher in KE (12 ± 12 on a 96-points scale) and KE+BIC (11 ± 9) compared to CON (7 ± 12) and BIC (5 ± 8). This resulted from increased distress at the systemic (dizziness) and upper abdominal (bloating, nausea, vomiting) level 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, while 9 out of 12 subjects correctly identified their best TT_{30'} performance.

DISCUSSION

It has been previously suggested that elevated blood β HB concentration due to ketone ester intake inhibits glycolytic flux during exercise (1). However, such effect did not emerge during prolonged (~3h) 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 ketone ester 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 NaHCO₃, elevated mean power output by ~5% during a 15-min TT at the end of a 3h simulated cycling race (10). However, this effect late in the event occurred at a time when circulating blood β HB concentration had reverted to <0.5mM after a 3-5 mM peak earlier in the exercise. This excludes the theory that β HB 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 co-ingestion of NaHCO₃ with KE, aiming to acutely raise blood β HB 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

which are typically recommended in the context of elite cycling time-trial performance. Exogenous ketosis was induced by ketone ester intake during warming up, which expectedly elevated blood β HB to $\sim$ 3-5 mM during the time-trial. In line with our earlier findings (10), NaHCO_3 co-ingestion was fully effective in preventing acidosis related to oral ketone ingestion. However, in contrast to our hypothesis, independent of whether NaHCO_3 was co-ingested, exogenous ketosis slightly impaired time-trial performance. The current data taken together with literature data (12, 13, 21) clearly indicate that acute ketone ester ingestion must be dissuaded in the context of short maximal endurance exercise events lasting shorter than 1h.

Previous studies have yielded equivocal results with regard to the effects of exogenous ketosis on exercise performance (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 β HB levels above 1 mM during exercise (1, 9–11). As a result, 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 time-trial preceded by a 1h 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 as well as 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 time-trial (13), and a 15 min time-trial at the end of a 3h 15min simulated cycling race (9, 10). Accordingly, in the conditions of the current study KE intake alone improved performance neither in TT_{30'} nor in 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 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 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 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 TT_{30'} in KE and KE+BIC correlated with the extent of blood β HB elevation by KE. This may also explain why a previous study found no effect of KE establishing low blood β HB (~1.5mM) on performance in a ~40 min endurance event (12). Conversely, in the current protocol subjects worked at 3-5mM circulating blood β HB 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 was very low, indeed, yet nonetheless slightly higher in the KE vs. 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 co-workers (28) showing a dose-dependent increase in gastro-intestinal discomfort following KE ingestion at rest.

High-dose ketone ester 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 appear to affect performance because power outputs in $\text{TT}_{30'}$ were independent of NaHCO_3 co-ingestion. At first glance this finding is in contrast with our recent report showing that co-ingestion of KE and NaHCO_3 increased 15 min time-trial performance at the end of a 3h 15min simulated cycling race (10). However, in the latter study blood ketone levels were only elevated during the initial 2 hours of submaximal exercise from a ~3h 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 above 3 mM during the performance measurements in $\text{TT}_{30'}$ 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 β HB 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^-), pre-exercise 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. first reported ketone ester intake to suppress blood lactate accumulation during exercise by inhibition of muscle glycogen breakdown (1). 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 (60g per h), KE failed to affect the exercise-induced increase in blood lactate during 3 hours of intermittent submaximal exercise at 60-90% of the lactate threshold (9). This is in line with other observations that KE does not alter changes in blood lactate concentration during moderate exercise intensities (11, 12). But discordant findings have been reported with regard to blood lactate accumulation during maximal exercise events. Acute exogenous ketosis due to ketone ester 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 β HB 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 $\text{TT}_{30'}$ but not during SPRINT. In fact, the effect of KE to suppress blood lactate increase during $\text{TT}_{30'}$ largely occurred during the initial half of the trial, where after further increments at higher blood lactate concentrations (>4-5 mM) were similar between KE

and CON. This may indicate that at low circulating blood lactate concentrations β HB inhibits lactate efflux from muscle cells via competitive inhibition of monocarboxylate transporters. But 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 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 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 TT_{30'}, conceivably was minor (6). It is probable that the lower plasma FFA concentrations during KE result largely, if not entirely, from β HB-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 3h 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 $[\text{HCO}_3^-]$ responses between these studies. In our earlier study, both blood pH and $[\text{HCO}_3^-]$ were higher in the KE+BIC compared to the control condition by the start of the 15-min TT following the 3h 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 $[\text{HCO}_3^-]$ at the start of $\text{TT}_{30'}$ were similar between KE+BIC and CON, which resulted in similar rates of HCO_3^- utilization during $\text{TT}_{30'}$. Increased vs. decreased extracellular HCO_3^- reserve stimulates vs. 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 ($[\beta\text{HB}] > 1$ 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 $[\beta\text{HB}]$ correlated with greater degree of performance impairment. Along such rationale, in line with our earlier observations (10), co-ingestion of NaHCO_3 enhanced the blood βHB response to KE (see figure 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 (see figure 4C).

KE, alone or combined with NaHCO_3 slightly impaired performance in $\text{TT}_{30'}$. 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 , since BIC alone also improved performance in SPRINT. This

finding is in line with numerous previous studies showing an ergogenic effect of NaHCO_3 during short high-intensity exercise (for reviews see (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 $[\text{HCO}_3^-]$ levels as 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 $[\text{Ca}^{2+}]$ and $[\text{Cl}^-]$ during a ~3h simulated cycling race following NaHCO_3 intake independent of KE co-ingestion (10). Yet, the NaHCO_3 -induced decrease in the concentration of both electrolytes was 50% smaller in the current compared to our previous study. Further research needs to elucidate the concerted actions of KE and NaHCO_3 on the intra- 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 non-metabolic 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 24h 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% even though 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^- co-ingestion. 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.

Conflicts of Interest and Source of Funding

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Author contributions

Conception and design of the study: CP and PH. Data collection: CP, FW and MR. Analysis and interpretation of the data and manuscript drafting: CP and PH. All authors critically evaluated the manuscript and approved for submission. All authors 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.

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FIGURE CAPTIONS

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 warm-up (WU) during which they cycled at low to moderate exercise intensities (50-70% of lactate threshold) alternated by 3 high-intensity efforts (2x 3 min at 100% of lactate threshold and 1x 1min at 120% of LT) while gradually ingesting 60g carbohydrates. Following WU, subjects performed a 30 min simulated cycling time-trial (TT_{30'}) and a ~1 min all-out cycling bout at 175% of the lactate threshold (SPRINT). Before and during the exercise protocol subjects received either placebo (CON), ketone ester (KE), bicarbonate (BIC) or ketone ester plus bicarbonate (KE+BIC). Intensities during WU were set relative to each individual's lactate threshold.

Figure 2. Effect of ketone ester intake, alone or combined with bicarbonate supplementation on blood D-βHB, glucose and lactate during the exercise protocol.

Data are mean $\pm$ SD ($n = 12$) for blood D-βHB (A), glucose (B) and lactate (C) concentrations. Subjects performed a 60 min warm-up (WU) followed by a 30 min simulated time-trial (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). Grey 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.

Figure 3. Effect of ketone ester and/or bicarbonate supplementation on blood pH and bicarbonate concentration.

Data are mean $\pm$ SD and represent blood pH (A) and bicarbonate (HCO_3^-) concentration (B). In a crossover design, subjects ($n = 12$) received control ($\text{--}\bigcirc\text{--}$, CON), ketone ester ($\text{--}\bigcirc\text{--}$, KE), bicarbonate ($\text{--}\bullet\text{--}$, BIC) or ketone ester and bicarbonate ($\text{--}\bullet\text{--}$, KE+BIC). Grey area depicts time zone during which subjects received the KE supplement. $\dagger P < 0.05$ 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).

Figure 4. Effect of ketone ester and/or bicarbonate supplementation on exercise performance.

Data are mean $\pm$ SD and represent average power output during the 30 min simulated time-trial ($\text{TT}_{30'}$) (A) and individual differences between KE and KE+BIC vs. CON and BIC together with mean $\pm$ 95% CI (B) as well as individual differences between each experimental condition compared to CON with mean $\pm$ 95% CI (C). Mean $\pm$ SD for time to exhaustion in the SPRINT (D), and individual differences between BIC and KE+BIC vs. CON and KE together with mean $\pm$ 95% CI (E) as well as individual differences between each experimental condition compared to CON with mean $\pm$ 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. $\dagger P < 0.05$ 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).

Figure 1

Figure 2

Figure 3

Figure 4

	-190 min Breakfast	-70 min Start WU	0 min Start TT_{30'}	30 min End TT_{30'}	49 min End SPRINT
Plasma [Ca²⁺]					
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

Table 1. Effect of ketone ester and/or bicarbonate supplementation on plasma electrolytes.

Values are mean ± SD in mM for plasma calcium (Ca²⁺), 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 ester and bicarbonate (KE+BIC). BIC supplementation was started at -190

min, whilst 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).

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Table 2. Effect of ketone ester and/or bicarbonate supplementation on rate of perceived exertion and heart rate.

	CON	KE	BIC	KE+BIC	Value
RPE (6-20)					es
WU	10.8 ± 1.0	11.0 ± 1.0	10.3 ± 1.0	10.8 ± 1.3	are
TT _{30'}	18.1 ± 0.8	17.4 ± 1.6	17.3 ± 0.9	17.4 ± 1.2	mea
SPRINT	18.8 ± 1.1	18.4 ± 1.1§	19.0 ± 0.6	18.4 ± 1.2§	n ±
HR (bpm)					SD
TT _{30'}	167 ± 14	168 ± 11	168 ± 14	168 ± 13	for
SPRINT	177 ± 14	176 ± 12	178 ± 13	179 ± 11	

rate of perceived exertion (RPE) at the end of WU, TT_{30'} and SPRINT. Heart rate (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 ester and bicarbonate (KE+BIC) before and during exercise. § P = 0.07 for effect of KE (KE and KE+BIC vs. CON and BIC).