No Benefit of Ingestion of a Ketone Monoester Supplement on 10-km Running Performance

MARK EVANS[1], FIONN T. MCSWINEY[2], AIDAN J. BRADY[1], and BRENDAN EGAN[1,3]

[1]School of Health and Human Performance, Dublin City University, Glasnevin, Dublin, IRELAND; [2]School of Health Sciences, Waterford Institute of Technology, Waterford, IRELAND; and [3]National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin, IRELAND

ABSTRACT

EVANS, M., F. T. MCSWINEY, A. J. BRADY, and B. EGAN. No Benefit of Ingestion of a Ketone Monoester Supplement on 10-km Running Performance. *Med. Sci. Sports Exerc.*, Vol. 51, No. 12, pp. 2506–2515, 2019. **Purpose:** Preexercise ingestion of exogenous ketones alters the metabolic response to exercise, but effects on exercise performance have been equivocal. **Methods:** On two occasions in a double-blind, randomized crossover design, eight endurance-trained runners performed 1 h of submaximal exercise at approximately 65% $\dot{V}O_{2max}$ immediately followed by a 10-km self-paced time trial (TT) on a motorized treadmill. An 8% carbohydrate-electrolyte solution was consumed before and during exercise, either alone (CHO + PLA), or with 573 mg·kg^{-1} of a ketone monoester supplement (CHO + KME). Expired air, HR, and RPE were monitored during submaximal exercise. Serial venous blood samples were assayed for plasma glucose, lactate, and β-hydroxybutyrate concentrations. **Results:** CHO + KME produced plasma β-hydroxybutyrate concentrations of approximately 1.0 to 1.3 mM during exercise ($P < 0.001$), but plasma glucose and lactate concentrations were similar during exercise in both trials. $\dot{V}O_2$, running economy, respiratory exchange ratio, HR, and RPE were also similar between trials. Performance in the 10-km TT was not different ($P = 0.483$) between CHO + KME (mean, 2402 s; 95% confidence interval, 2204–2600 s) and CHO + PLA (mean, 2422 s; 95% confidence interval, 2217–2628 s). Cognitive performance, measured by reaction time and a multitasking test, did not differ between trials. **Conclusions:** Compared with carbohydrate alone, coingestion of KME by endurance-trained athletes elevated plasma β-hydroxybutyrate concentrations, but did not improve 10-km running TT or cognitive performance. **Key Words:** ATHLETES, β-HYDROXYBUTYRATE, COGNITION, ENDURANCE, LACTATE, TIME TRIAL

The therapeutic and performance potential of exogenous ketone supplements has been the subject of increasing interest in recent years (1,2). Metabolic effects of the ketone bodies (KB), namely, β-hydroxybutyrate (βHB) and acetoacetate, are well established in many organs, including attenuation of glycolysis, hepatic glucose output, and adipose tissue lipolysis (3), but their potential role in modulating substrate utilization has garnered attention for athletic performance (4,5). In the fasted state, KB provide up to 10% of energy to skeletal muscle during exercise (6), and after acute ingestion of exogenous ketone supplements, this contribution can apparently increase to 16% to 18% when circulating βHB is elevated to the 3 to 4 mM range (5). Moreover, this increase in βHB oxidation coincides with a reduction in glycolytic flux, as evidenced by an attenuation in the exercise-induced rise in plasma lactate and glycolytic intermediates, and an increase in intramuscular triglyceride utilization during exercise (5).

Circulating KB concentrations are <0.1 mM in the postprandial state, whereas hyperketonemia is accepted as KB concentrations exceeding 0.2 mM (3). Ingestion of a variety of exogenous ketone supplements can acutely produce nutritional ketosis (4,5,7–17), which has been defined as circulating KB concentrations >0.5 mM (18). The most potent of these exogenous ketone supplements is the (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (KME). When ingested at rest in the fasted state, KME produces a dose-dependent increase in circulating βHB concentrations of up to 6 mM 20 min after the ingestion of up to 573 mg·kg^{-1} body mass (5,9). This elevation in βHB concentration coincides with decreases in plasma glucose, free fatty acids, triglycerides,

Address for correspondence: Brendan Egan, Ph.D., School of Health and Human Performance, Dublin City University, Glasnevin, Dublin 9, Ireland; E-mail: brendan.egan@dcu.ie.
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and ghrelin concentrations (5,8,9,13). Exercise attenuates the rise in βHB concentrations, as ingestion of 573 mg·kg^{-1} KME before 45-min cycling at 45% and 75% peak power output (W_{max}) resulted in circulating βHB of approximately 4.0 mM and approximately 3.0 mM, respectively. As a consequence of the aforementioned effects on substrate utilization, acute ingestion of KME attenuates the rise in plasma glucose and lactate concentrations during exercise, whether in an endurance cycling or intermittent running context (4,5).

These metabolic consequences have been proposed to explain the observation that the coingestion of KME in addition to a carbohydrate-based fueling strategy improved performance in a 30-min maximum distance cycling time trial (TT) by 2% when preceded by 1 h of submaximal "preload" exercise (5). In contrast, high-intensity shuttle running capacity (~4 to 6 min) performed after 75 min of intermittent running was not improved in team sport athletes with KME coingestion compared with carbohydrate alone (4). Although the former study considered a "sparing" of muscle glycogen to be a major factor in the performance benefit (5), the latter study speculated that the attenuation of glycolytic flux in the presence of elevated circulating βHB may have been a factor in the lack of performance benefit in that exercise model (4). Performance in exercise of long duration that incorporates high intensity efforts (i.e., sprint finishes, climbs) is largely dependent on carbohydrate utilization (19). Therefore, nutrition strategies that could spare muscle glycogen and maintain high intensities in the latter parts of races are of interest to scientists and practitioners (20). However, if glycogen sparing occurs via an attenuation of glycolytic flux that cannot be overcome when higher intensity efforts are required, this would instead be likely to impair performance (19). Moreover, the recent observation that acute ingestion of KME before intermittent exercise in team sport athletes resulted in preserved executive function as measured by a decision making task after volitional exhaustion (4) remains to be confirmed in other exercise settings.

Therefore, the aim of the present study was to investigate the effects of acute ingestion of an exogenous ketone supplement in the form of a commercially available KME on physiological responses, and physical and cognitive performance in endurance-trained runners in response to 1 h submaximal exercise immediately followed by a 10-km TT.

METHODS

Participants. Eight trained, middle and long distance runners (male/female, 7/1; age, 33.5 ± 7.3 yr; height, 1.79 ± 0.07 m; body mass, 68.8 ± 9.7 kg; body fat, 8.0% ± 4.1%; $\dot{V}O_{2max}$, 62.0 ± 5.6 mL·kg^{-1}·min^{-1}) gave written informed consent to participate after written and verbal explanations of the procedures. Ethical approval (permit number: DCUREC2018_039) was obtained from the Dublin City University Research Ethics Committee in accordance with the Declaration of Helsinki.

Experimental design. Participants visited the laboratory for exercise tests on four separate occasions over a 21- to 28-d period, comprising one baseline, one familiarization, and two main experimental trials. During their first visit to the laboratory, each participant's maximal rate of oxygen consumption ($\dot{V}O_{2max}$) was determined using an incremental treadmill test to volitional exhaustion. The exercise protocol performed in the familiarization visit (visit 2) and two main experimental trials (visits 3 and 4) comprised of a preload of 1 h of treadmill running at 65%$\dot{V}O_{2max}$ followed by a self-paced 10-km TT performance test performed on a motorized treadmill (Fig. 1). A battery of cognitive tests was performed before and after the exercise protocol. The main experimental trials were performed in a double-blind, placebo-controlled, randomized crossover design. Visits 2, 3, and 4 were identical in terms of the pretest preparation (standardized physical activity and diet for 24 h before each visit) and the exercise protocol. The visits differed only in the drinks consumed before and during exercise, namely an 8% carbohydrate-electrolyte solution, which was coingested with either a flavored placebo condition (CHO + PLA), or included the (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (CHO + KME). The primary outcome was endurance performance measured by time to complete the self-paced 10-km TT, with secondary outcomes including cognitive performance, oxygen consumption

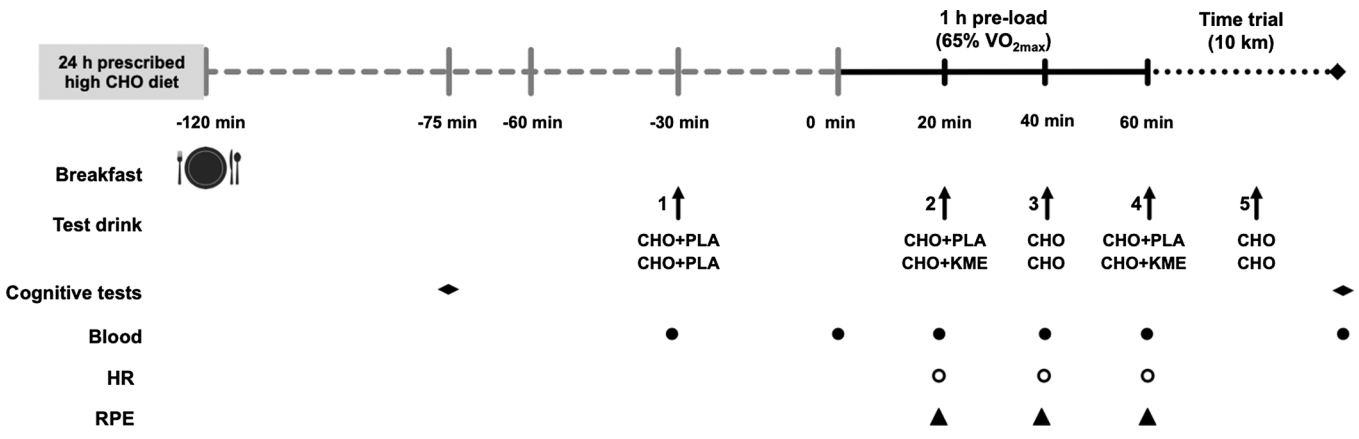

FIGURE 1—Schematic of the study protocol.

($\dot{V}O_2$), running economy, RER, HR, RPE, and plasma βHB, glucose, and lactate concentrations.

Assessment of $\dot{V}O_{2max}$ and submaximal running speeds. Body mass was measured to the nearest 0.2 kg using a calibrated digital scales (SECA, Hamburg, Germany), and height was measured to the nearest 0.01 m using a wall-mounted stadiometer (Holtain, Crymych, UK). Body fat was determined by bioelectrical impedance analysis (DC-430 U Dual Frequency Analyzer; Tanita, Arlington Heights, IL). All exercise testing and experimental trials were conducted on a motorized treadmill (T200; COSMED, Rome Italy). Initially, for the determination of the responses in $\dot{V}O_2$ and blood lactate concentration at submaximal running speeds, participants ran for 4-min stages at progressively increasing speeds, interspersed with a 1-min rest interval for determination of blood lactate concentrations (Lactate Pro 2; Arkray, Kyoto, Japan), RPE (Borg scale), and HR (Polar H7; Polar, Kempele Finland). The first stage was 4 $km \cdot h^{-1}$ slower than the average speed corresponding to each participant's personal best time for a 10-km race. For each subsequent stage, the running speed was increased by 1 $km \cdot h^{-1}$ until the running speed exceeded the speed corresponding to their personal best 10-km race speed. After a 10-min rest, participants began running at a speed corresponding to the last completed speed of the preceding test. Treadmill speed was increased by 2.0 $km \cdot h^{-1}$ every 2 min for two stages, after which treadmill gradient was increased by 1.0% every 1 min until volitional fatigue. Expired air was collected and analyzed throughout these tests using the Quark RMR metabolic cart (COSMED). $\dot{V}O_2$, carbon dioxide production (VCO_2), and RER were calculated from an average of breath-by-breath measurements during the last 30 s of each stage during the submaximal running stages and the assessment of $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ was considered to have been achieved if two of the following criteria were achieved: (i) plateauing of $\dot{V}O_2$ despite increasing treadmill speed (increase in $\dot{V}O_2$ of less than 2.0 $mL \cdot kg^{-1} \cdot min^{-1}$), (ii) HR within 5% of the age-predicted HR_{max} ($208 - 0.7 \times$ age in years), and (iii) an RER ≥1.10.

Cognitive test battery. The battery of cognitive tests (CANTAB Cognition, Cambridge, UK) was administered via a touch screen tablet lasting approximately 10 min. An identical test battery was administered before and after each trial in visits 2, 3, and 4.

During the reaction time (RTI) test, participants select and hold a button at the bottom of the screen and five circles are presented above. In each case, a yellow dot appears in one of the five circles, and the participants must react as soon as possible, releasing the button at the bottom of the screen, and selecting the circle in which the dot appeared. Release time (ms), reaction time (ms), and number of errors were recorded.

The multitasking test (MTT) is a test of executive function that measures the participant's ability to switch attention between stimuli, and ignore task-irrelevant information. White arrows are displayed on a black background, with the arrows located on either the left or right side of the screen, and pointing either to the left or to the right. A cue is displayed at the same time as the arrows, reading either "SIDE" or "DIRECTION." When the "SIDE" cue is presented, the participant is required to press a button on the left or right of the screen corresponding to the side of the screen where the arrow is presented, regardless of the direction the arrow is pointing. Conversely, when the "DIRECTION" cue is presented, the participants are required to touch a button on the left or right of the screen corresponding to the direction the arrow is pointing, regardless of which side of the screen the arrow is presented. Reaction time (ms), and number of correct and incorrect responses were recorded.

Pretrial preparation. All experimental trials commenced between 7:30 AM and 11:30 AM, and were completed within a period of 4.0 to 4.5 h (Fig. 1). On an individual basis, participants performed their second main experimental trial at the same time ±1 h as their first main trial. Pretrial preparation was the same for the familiarization visit and each main experimental trial. Participants were asked to abstain from alcohol for 48 h and caffeine for 24 h, and refrain from strenuous exercise training on the day before each trial. For the day before experimental trials, participants were provided with a prescribed meal plan that provided approximately 2800 kcal (~41 $kcal \cdot kg^{-1}$) at a macronutrient ratio of 60% carbohydrate (~6.2 $g \cdot kg^{-1}$), 20% protein, and 20% fat. Participants performed the two main experimental trials separated by either 7 or 14 d.

Main experimental trials. The protocol for the familiarization and main experimental trials were identical except for the drinks consumed before and during exercise (Fig. 1). Participants arrived to the laboratory in a fasted state 2 h before the commencement of exercise, and immediately consumed a standardized breakfast of quick-cook porridge oats and cereal bars providing approximately 300 to 400 kcal (~4.4–5.8 $kcal \cdot kg^{-1}$) and approximately 1.0 $g \cdot kg^{-1}$ of carbohydrate, and 500 mL of water. Participants proceeded to complete the cognitive test battery 45 min after breakfast. Thereafter, an indwelling catheter (21G Insyte Autoguard; Becton Dickinson, Franklin Lakes, NJ) was introduced into an antecubital vein for serial blood sampling at rest (−30 and 0 min), during submaximal exercise (20, 40, and 60 min) and immediately after the 10-km TT.

For each trial, a bolus of a given drink was ingested 30 min before exercise (drink 1), at 20 min intervals during the 1 h of submaximal running (drinks 2 to 4), and at the 5-km mark of the 10-km TT (drink 5) (Fig. 1). The carbohydrate-based fueling strategy (CHO) consisted of a 6.4% carbohydrate-electrolyte solution (Lucozade Sport; Lucozade Ribena Suntory Ltd., Uxbridge, UK) with maltodextrin (Cargill Inc, Minneapolis, MN) added to make an 8.0% carbohydrate-electrolyte solution that was provided at a rate of approximately 1.0 $g \cdot min^{-1}$ of exercise. During CHO + PLA, CHO was supplemented with denatonium benzoate, malic acid and arrow root extract to mimic the bitter taste and mouth-feel of the KME. During CHO + KME, CHO was supplemented with 573 $mg \cdot kg^{-1}$ body mass of a (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (HVMN™ Ketone; HVMN, Inc., San Francisco, CA). The commercially available ketone ester was mixed directly with the carbohydrate-electrolyte

solution for ingestion, and the 573 mg·kg^{-1} dose was divided into three boluses at a ratio 50:25:25 ingested at −30 min (drink 1), 20 min (drink 2), and 60 min (drink 4), respectively (Fig. 1). During CHO + PLA, drinks 1, 2, and 4 were flavored with the bitter additives to taste match with CHO + KME, and in both trials, drinks 3 and 5 were provided as the unadulterated 8% carbohydrate-electrolyte solution. All drinks were administered in opaque drinks bottles.

For the exercise protocol, participants first performed a standardized 5 min warm-up on the motorized treadmill (8 km·h^{-1}) followed by self-selected stretching. Participants then performed 1 h of treadmill running at a speed corresponding to approximately 65% $\dot{V}O_{2max}$ (Table 1). Immediately after completion of the 1-h preload, participants completed a 10-km TT. The preload followed by TT protocol was modeled on the previous work demonstrating a benefit of KME on cycling TT performance (5) and has been similarly applied to treadmill running in previous studies (21,22). Before each TT, participants were told to complete the distance as fast as possible, that is, to race the 10-km. They were allowed to adjust the treadmill speed as often and by as much as desired by manually adjusting a side-mounted control panel on the treadmill. Increments or decrements in speed were 0.1 km·h^{-1} in response to each press of an up or down arrow button, respectively. The 10-km TT began with the participant accelerating from a standing start. Participants were blinded to the speed of the treadmill and the time elapsed at all times, but were aware of the distance covered throughout the TT, including the 5-km mark when drink 5 was provided. After completing the 10-km TT, participants completed the same cognitive test battery as completed before exercise.

Venous blood samples were collected at 30 min before exercise, at 20-min intervals during submaximal exercise, and immediately after the 10-km TT. HR and RPE were recorded at 20-min intervals during submaximal exercise. Expired air was collected during the first 10 min, 25 to 30 min, and 55 to 60 min of the submaximal exercise for the monitoring of exercise intensity, and calculation of RER and running economy. Running economy is expressed as the volume of oxygen required to run 1 km relative to body mass (mL·kg^{-1}·km^{-1}) (23). Incidences of gastrointestinal (GI) symptoms were recorded by interview after each trial. At the end of visit 4, participants completed an exit interview in which they were asked whether they could identify the CHO + KME condition and to identify which experimental trial they believed that they performed their best TT.

Blood analysis. Blood was collected in plastic tubes (2 mL) containing sodium heparin (Plus Blood Collection Tubes; Becton Dickinson) for subsequent analysis of βHB. A second blood sample was collected in plastic tubes (4 mL) containing sodium fluoride (Plus Blood Collection Tubes; Becton Dickinson) for subsequent analysis of glucose and lactate. All collection tubes were prechilled, and blood samples were stored on ice before centrifugation at 3000*g* for 10 min at 4°C, after which aliquots of plasma were separated for storage at −80°C until later analysis. Plasma βHB was determined by colorimetric assay as per the manufacturer's instructions (MAK041; Sigma-Aldrich, Arklow, Ireland). Plasma glucose and lactate were measured using the RX Daytona™ chemical autoanalyzer and appropriate reagents as per the manufacturer's instructions (Randox Laboratories, Crumlin, UK: assay codes GL3815 and LC3980, respectively).

Statistical analysis. The required sample size was calculated *a priori* using performance in the 10-km TT as the primary outcome measure. Based on the reliability data for the preloaded 10-km TT protocol employed (22), the assessment of other running TT protocols (24), and the variability of real-world performance in races of similar distance (25), we

TABLE 1. Physiological responses to 1 h of treadmill running at approximately 65%$\dot{V}O_{2max}$ when carbohydrate was coingested with either placebo (CHO + PLA) or a ketone monoester (CHO + KME).

	Time			
	0–10 min	10–30 min	30–60 min	*P*
Running speed (km·h^{-1})	12.4 (11.3–13.5)	12.4 (11.3–13.5)	12.3 (11.1–13.5)	
$\dot{V}O_2$ (L·min^{-1})				Time, $P = 0.517$
CHO + PLA	2.84 (2.52–3.16)	2.84 (2.56–3.12)	2.81 (2.53–3.09)	Condition, $P = 0.153$
CHO + KME	2.78 (2.42–3.13)	2.79 (2.42–3.13)	2.72 (2.49–2.95)	Interaction, $P = 0.700$
%$\dot{V}O_{2max}$				Time, $P = 0.576$
CHO + PLA	67.0 (62.8–71.2)	66.9 (64.5–69.4)	66.2 (63.8–69.4)	Condition, $P = 0.170$
CHO + KME	65.3 (60.9–69.8)	65.8 (62.6–69.8)	64.1 (63.2–65.0)	Interaction, $P = 0.710$
Running economy (mL·kg^{-1}·km^{-1})				Time, $P = 0.633$
CHO + PLA	202 (184–219)	203 (185–220)	202 (185–219)	Condition, $P = 0.182$
CHO + KME	196 (181–212)	199 (181–217)	196 (179–213)	Interaction, $P = 0.779$
$\dot{V}CO_2$ (L·min^{-1})				Time, $P = 0.058$
CHO + PLA	2.67 (2.36–2.99)	2.60 (2.30–2.90)	2.55 (2.26–2.84)	Condition, $P = 0.470$
CHO + KME	2.63 (2.28–2.98)	2.58 (2.28–2.89)	2.50 (2.26–2.74)	Interaction, $P = 0.677$
RER				Time, $P < 0.001$*
CHO + PLA	0.94 (0.92–0.96)	0.91 (0.89–0.94)	0.91 (0.88–0.93)	Condition, $P = 0.315$
CHO + KME	0.95 (0.92–0.97)	0.92 (0.89–0.96)	0.92 (0.89–0.95)	Interaction, $P = 0.478$
HR (bpm)				Time, $P = 0.121$
CHO + PLA	141 (133–149)	146 (137–155)	145 (137–154)	Condition, $P = 0.359$
CHO + KME	140 (131–150)	144 (134–154)	143 (134–152)	Interaction, $P = 0.747$
RPE				Time, $P < 0.001$*
CHO + PLA	10 (9–12)	11 (10–13)	12 (10–13)	Condition, $P = 0.903$
CHO + KME	10 (8–12)	11 (9–12)	11 (9–13)	Interaction, $P = 0.656$

Data are presented as mean (95% CI), $n = 8$. *$P < 0.001$.

estimated a coefficient of variation of 1.5% for performance in the 10-km TT. We aimed to detect a 2.5% change in 10-km TT performance based on the smallest worthwhile difference (SWD) described by Russell et al. (22) for this preloaded 10-km TT protocol being 2.1%. Consequently, the sample size calculation at an α level of 0.05 and power (1 − β) of 0.8 revealed that six participants would be sufficient to detect a 2.5% change in 10-km TT performance. However, considering the adequacy of sample sizes in similar studies, as a conservative measure we recruited a final sample size of $n = 8$. Data were evaluated using Prism v8.0 (GraphPad Software, Inc., San Diego, CA) and are presented as mean [lower, upper 95% confidence interval (CI) of the mean], except for the participant characteristics, which are described as mean ± SD. A one-way repeated-measures ANOVA was used to determine whether a trial order effect existed across visits 2, 3, and 4 in the time to complete the 10-km TT. A paired samples t-test was used to determine differences between trials in time to complete the 10-km TT. The SWD was set at 0.2 between-subject SD, which is suggested to represent a practically relevant change in performance in athletes. Thus, the SWD corresponded to 48 s, or 2.0%, for 10-km TT performance in this study. Two-way (time–condition) repeated-measures ANOVA was used to determine differences between the two experimental trials for all variables with serial measurements. When a main effect of condition, or an interaction effect between condition and time was indicated, *post hoc* testing was performed with Bonferroni's correction with multiplicity-adjusted *P* values applied to compare CHO + KME to CHO + PLA at the respective time points. The data were tested for normality using Shapiro–Wilk test before proceeding with the parametric tests described. For null hypothesis statistical testing, the significance level was set at $\alpha = 0.05$ for all tests.

RESULTS

Plasma βHB, glucose, and lactate concentrations. Postprandial plasma concentrations of βHB (mean [95% CI]: CHO + KME, 0.27 [0.22–0.33] mM; CHO + PLA, 0.28 [0.14–0.43] mM), glucose (CHO + KME, 3.96 [3.22–4.70] mM; CHO + PLA, 3.70 [3.06–4.35] mM), and lactate (CHO + KME, 1.04 [0.79–1.29] mM; CHO + PLA, 1.02 [0.84–1.20] mM) did not differ between trials (all $P > 0.99$). A main effect of time and condition (both $P < 0.001$) and a time–condition interaction effect ($P < 0.001$) were observed for plasma βHB concentrations (Fig. 2A). Ingestion of CHO + KME resulted in a rise in plasma βHB concentrations to 0.99 (0.85–1.14) mM at 0 min. βHB concentrations peaked at 1.33 (1.13–1.52) mM during submaximal exercise at 40 min, with similar concentrations observed at the cessation of the 10-km TT at 1.33 (0.95–1.70) mM.

A main effect of time ($P < 0.001$) and condition ($P = 0.027$) was observed for plasma glucose concentrations (Fig. 2B). Plasma glucose concentrations were lower in CHO + KME at 0 min, that is, 30 min after ingestion of the first bolus of

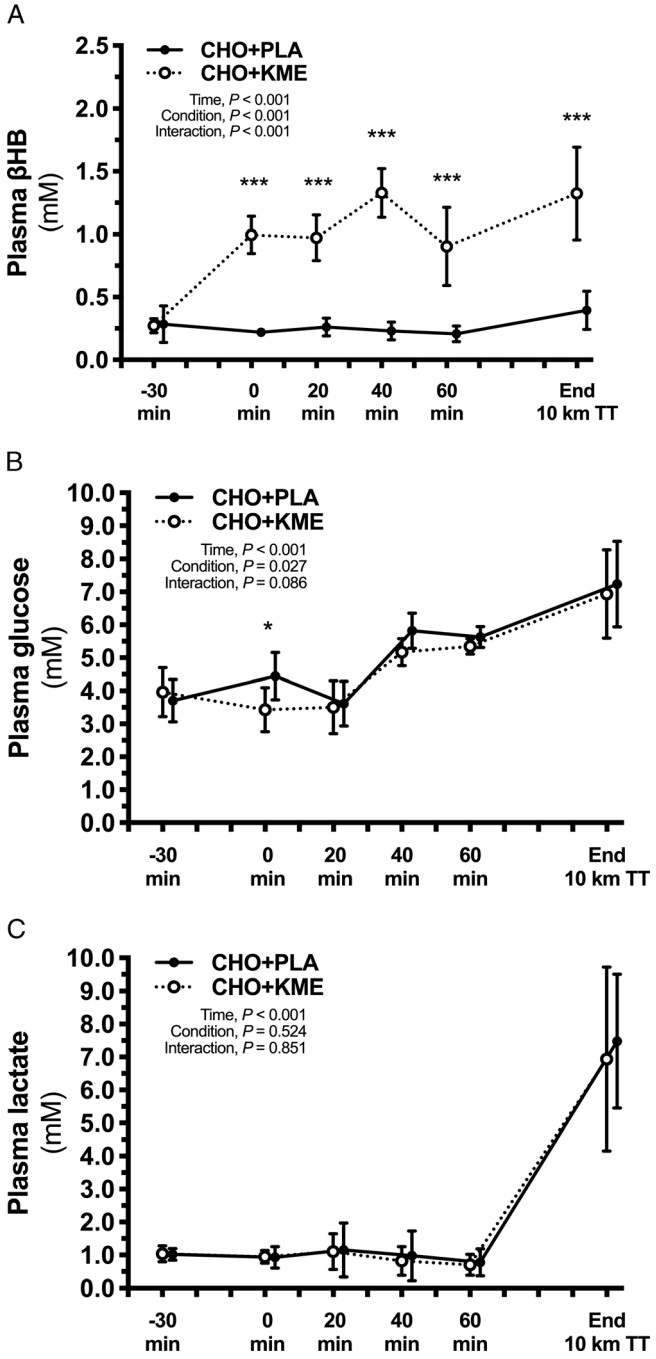

FIGURE 2—Plasma βHB (A), glucose (B), and lactate (C) concentrations during each trial. Data are presented as mean values, with error bars representing 95% CI. *$P < 0.05$ for CHO + KME vs CHO + PLA; ***$P < 0.001$ for CHO + KME vs CHO + PLA.

either CHO + KME or CHO + PLA (CHO + KME, 3.87 [3.22–4.70] mM; CHO + PLA, 4.52 [3.91–5.13] mM; $P = 0.016$) (Fig. 2B). Plasma glucose concentrations rose throughout submaximal exercise (Fig. 2B) with the highest concentrations observed at cessation of the 10-km TT (CHO + KME, 6.94 [5.60–8.28] mM; CHO + PLA 7.24 [5.93–8.54] mM), with no difference between trials ($P > 0.99$).

A main effect of time ($P < 0.001$) was observed for plasma lactate concentrations, but were similar between trials

at all timepoints (Fig. 2C). Peak plasma lactate concentrations were observed at cessation of the 10-km TT (CHO + KME, 6.94 [4.15, 9.73] mM; CHO + PLA, 7.48 [5.46–9.51] mM; $P = 0.738$).

Submaximal exercise. Running speeds were identical between trials as per the study design. There was no difference in $\%\dot{V}O_{2max}$, $\dot{V}O_2$, running economy, VCO_2, RER, HR, and RPE between CHO + KME and CHO + PLA during the submaximal exercise period (Table 1). Main effects of time were observed for the decline in RER ($P < 0.001$), and the increase in RPE ($P < 0.001$) during the submaximal exercise bout (Table 1).

10-km TT performance. No trial order effect was observed for 10-km TT performance between visit 2 (2388 [2187–2588] s), visit 3 (2415 [2223–2607] s), and visit 4 (2409 [2197–2621] s) ($P = 0.742$). There was no statistically significant difference (−20 [−86–45] s; $P = 0.483$) in 10-km TT performance between trials (CHO + KME, 2402 [2204–2600] s; CHO + PLA, 2422 [2217–2628] s) (Fig. 3A). Compared to CHO + PLA, three participants demonstrated improvements in performance with CHO + KME that were greater than the SWD, and one participant demonstrated a decrement in performance with CHO + KME that was greater than the SWD (Fig. 3B). The remaining participants' differences in performance between trials were less than the SWD. Running speeds for each 2-km split during the 10-km TT did not differ between trials, but did increase progressively throughout the TT (main effect of time, $P < 0.001$) (Fig. 3C).

Cognitive performance. In the RTI test, main effects of time ($P = 0.026$) and condition ($P = 0.026$) were observed for release time, but no interaction effect was present ($P = 0.535$), whereas an interaction effect was observed for reaction time ($P = 0.014$) (Table 2). In the MTT, a main effect of time was observed for response latency ($P = 0.010$), correct responses ($P = 0.049$) and incorrect responses ($P = 0.036$), but no main effects of time, or interaction effects were observed across these parameters (all $P > 0.05$) (Table 2). Overall, there was no difference in cognitive performance between conditions in either the RTI, or MTT assessments (Table 2).

Gastrointestinal symptoms. Of the eight participants, 4 (50%) reported symptoms of GI distress during CHO + PLA and comprised 4 (50%), 3 (38%), 1 (13%), 1 (13%), and 1 (13%) incidences of belching, flatulence, reflux, urge to defecate, and diarrhea, respectively. Of the eight participants, 5 (63%) reported symptoms of GI distress during CHO + KME and comprised 3 (38%), 2 (25%), 1 (13%), 1 (13%), 1 (13%), and 1 (13%) incidences of belching, urge to defecate, cramps, reflux, nausea, and stitch, respectively.

Identification of CHO + KME and best performance trials. Of the eight participants, 2 (25%) correctly identified the trial in which they received CHO + KME, identifying CHO + KME by taste and a perceived alteration of performance. Six (75%) participants declared that they could not differentiate between CHO + PLA and CHO + KME. Seven (88%) participants correctly identified the trial in which they performed their best 10-km TT.

DISCUSSION

The present study investigated whether the acute ingestion of a commercially available ketone monoester supplement altered metabolic responses, and physical and cognitive performance in endurance-trained runners in response to 1 h of submaximal exercise immediately followed by a treadmill-based self-paced 10-km TT. Compared with placebo (CHO + PLA), ingestion of the ketone monoester (CHO + KME) elevated plasma βHB to approximately 1.0 mM at the onset of submaximal exercise, and reached approximately 1.3 mM at the end of the 10-km TT. However, CHO + KME did not alter the metabolic or cardiorespiratory responses to exercise, or demonstrate benefit to physical or cognitive performance compared to CHO + PLA ingestion.

The present study adds to the growing body of literature investigating the effects on exercise performance of elevating KB concentrations by exogenous means. The term "exogenous ketone supplement" encompasses a range of different forms of supplements, with each having differential effects

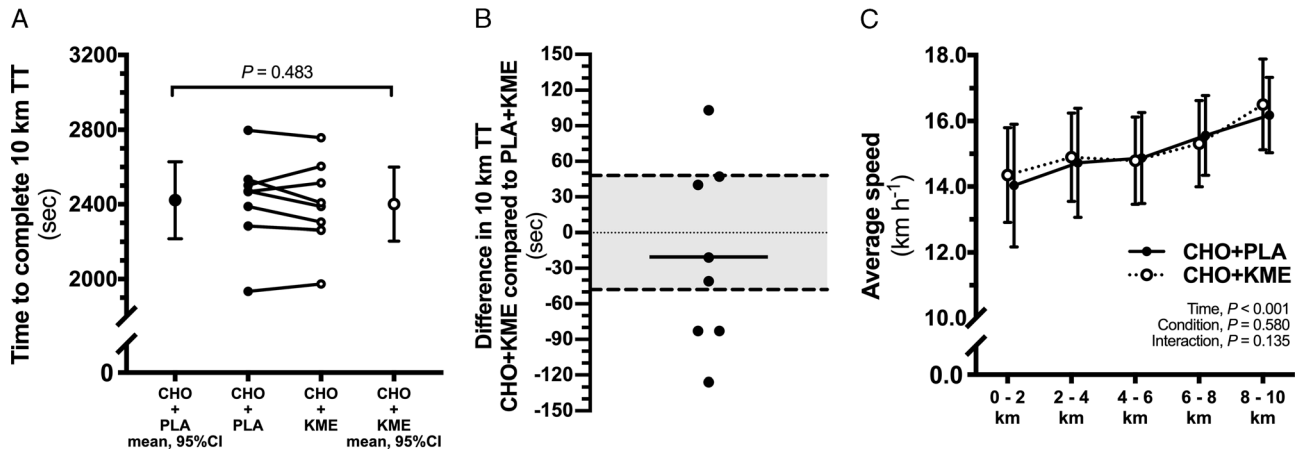

FIGURE 3—10-km TT performance (A), individual differences between CHO + KME compared to CHO + PLA (B), and running speeds for each 2 km split during the 10-km TT (C). Data in (A) and (C) are presented as mean values, with error bars representing 95% CI. The shaded area in (B) represents the range for the smallest worthwhile difference in 10-km TT performance in this cohort.

TABLE 2. Measures of cognitive performance assessed before and after each trial consisting of 1 h of treadmill running at approximately 65% $\dot{V}O_{2max}$ followed by a 10-km TT during which carbohydrate was coingested with either placebo (CHO + PLA) or a ketone monoester (CHO + KME).

	RTI							
	Release Time*,** (ms)			Reaction Time*** (ms)			Errors	
	Pre	Post	Post–Pre	Pre	Post	Post–Pre	Pre	Post	Post–Pre
CHO + PLA	417 (373 to 461)	401 (356 to 446)	−16 (−35 to 2)	223 (171 to 276)	221 (165 to 278)	−2 (−18 to 14)	0.3 (−0.1 to 0.6)	0.6 (0.0 to 1.2)	0.4 (−0.4 to 1.1)
CHO + KME	430 (383 to 477)	409 (368 to 450)****	−21 (−40 to −2)	214 (176 to 252)	232 (183 to 282)****	18 (2 to 34)	0.6 (−0.3 to 1.5)	0.5 (0.0 to 1.1)	−0.1 (−1.3 to 1.1)
	MTT							
	Response Latency* (ms)			Correct Responses*			Incorrect Responses*		
	Pre	Post	Post–Pre	Pre	Post	Post–Pre	Pre	Post	Post–Pre
CHO + PLA	599 (500 to 698)	561 (447 to 674)*****	−38 (−58 to −18)	159 (157 to 160)	157 (155 to 159)	−2 (−4 to 1)	1 (0 to 3)	3 (1 to 5)	2 (−1 to 4)
CHO + KME	583 (513 to 653)	541 (461 to 622)*****	−41 (−62 to −21)	158 (157 to 160)	157 (156 to 159)	−1 (−4 to 2)	2 (0 to 3)	3 (1 to 4)	1 (−2 to 4)

Data are presented as mean (95% CI), $n = 8$. Symbols are * $P < 0.05$ for main effect of time; ** $P < 0.05$ for main effect of condition; *** $P < 0.05$ for time–condition interaction effect; **** $P < 0.05$ for Post vs Pre; ***** $P < 0.001$ for Post vs Pre.

on the metabolic response to exercise, and exercise performance. These studies have included the acute ingestion of a (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (KME) (4,5), and a R,S-1,3-butanediol acetoacetate ketone diester (KDE) (12), racemic ketone salts (KS) (7,10,11,14), and the ketogenic compound 1,3-butanediol (BD) (21,26) before and/or during an exercise challenge. One of the key metabolic consequences of ingesting exogenous ketone supplements is the elevation in circulating βHB, but we speculate that exercise performance is unlikely to be affected unless βHB concentrations exceed 1.0 mM (27). To date, the only supplement to consistently exceed this threshold before an exercise challenge is the KME supplement (4,5). The KS and KDE elevate βHB concentrations into the 0.3 to 0.6 mM range (7,10,12), and ingestion of BD elevates βHB concentrations into the 0.6 to 0.8 mM range (21,26).

Specifically focusing on KME ingestion and exercise studies, ingestion of 573 mg·kg^{-1} of KME in the fasted state elevated βHB concentrations to approximately 2.0 mM 20 min after ingestion where it remained throughout 1 h cycling exercise at 75% W_{max} and a subsequent 30 min TT (5). In the fed state, ingestion of 750 mg·kg^{-1} of KME elevated βHB concentrations to >1.5 mM after 15 min of exercise, and approximately 2.6 mM by the end of 75 min of intermittent running followed by a short duration shuttle run to exhaustion (4). In contrast to this previous work, plasma βHB concentrations in the present study were elevated to approximately 1.3 mM during the exercise protocol, which is lower than previously observed at the same 573 mg·kg^{-1} dose (5). These previous studies have used a split dosing strategy to achieve the total KME dose described (4,5), and therefore we employed the same approach. The presently observed attenuated rise in plasma βHB concentrations compared to these studies is unsurprising given that ingestion of KME in the fasted state consistently elevates circulating βHB to >3.0 mM (8,9), whereas ingestion of KME in the postprandial state results in circulating βHB in the range from approximately 1.0 to 2.5 mM (4,5,9). For instance, ingestion of 395 mg·kg^{-1} in the fasted state produces peak βHB concentrations of approximately 3.0 mM but only approximately 2.0 mM in the fed state, a 33% reduction in C_{max} and coincides with a 27% reduction in 4 h βHB AUC in resting participants (9). Given that our participants were fed a lower initial dose of KME of 287 mg·kg^{-1}, that this ingestion occurred in a postprandial state, and that exercise commenced 30 min later, it is not surprising that we observed lower βHB concentrations before and during exercise compared to previous work (4,5).

Therefore, although the present protocol achieved acute nutritional ketosis, a benefit to endurance performance was not observed. This finding is consistent with a number of studies that have failed to find a performance benefit of exogenous ketone supplements in various exercise models (4,10–12,14). The variety of exogenous ketones supplements used, the large range of changes in circulating βHB produced, and a lack of consistency in the nutrients coingested and type of exercise challenge performed, make it difficult to make broad conclusions on the

efficacy of these supplements. However, only one study to date has demonstrated a performance benefit with the ingestion of KME, which when coingested with CHO increased the distance covered in a 30-min cycling TT by approximately 2% (mean ± SEM, 411 ± 162 m; $n = 8$), when preceded by 1 h preload exercise at 75% W_{max} (5). The proposed mechanism for this improvement in performance was a shift in the contribution to energy provision from substrate utilization of carbohydrate to fat, as demonstrated by reduction in glycolytic flux resulting in a "sparing" of muscle glycogen, and a concomitant increase in intramuscular triglyceride utilization during exercise (5).

The mechanistic basis whereby elevated ketones reduce carbohydrate utilization during exercise is likely an attenuation of glycolytic flux via an inhibition of pyruvate dehydrogenase and phosphofructokinase by increases in NADH:NAD^+, acetyl-CoA:CoA, or citrate. A reduction in glycolytic flux has been proposed to explain the attenuated exercise-induced rise in plasma lactate observed in previous studies providing KME (4,5). This attenuation was approximately 50% during 60 min at 75% W_{max} and 30 min TT in trained cyclists (5), and approximately 10% to 30% during 75 min of intermittent running in team sport athletes (4). However, no differences in plasma lactate were observed between trials in the present study either during the preload or TT periods. The submaximal exercise intensity of approximately 65% $\dot{V}O_{2max}$ employed was below lactate threshold for all participants, and therefore an intensity too low to observe an attenuation, if any, of the exercise-induced rise in plasma lactate. However, plasma βHB concentrations were elevated >1.0 mM before and at the cessation of the 10-km TT, yet no difference in plasma lactate was observed between trials.

Similarly, although a glucose-lowering effect of KME ingestion is well-documented whether ingested alone (5,8,9), or coingested with carbohydrate or protein (4,5,9,13,15), we observed an attenuation in the rise in plasma glucose concentrations only at 30 min after ingestion of the first bolus of CHO + KME compared with CHO + PLA. This difference in plasma glucose between trials was absent during the submaximal exercise period, and upon completion of the 10-km TT. When effects of KME ingestion on plasma glucose have been observed, the mechanism proposed has been an attenuation of hepatic gluconeogenesis and an increase in hepatic glucose uptake (13). Under certain conditions, elevated KB concentrations may have an insulinotropic action (6), but this is not always observed (28,29). When coingested with carbohydrate and/or protein, the effect of exogenous ketones to attenuate postprandial glycemia occurs despite similar circulating insulin concentrations between conditions (5,13,15).

We propose that the lack of differences between trials for plasma glucose and lactate, in contrast to previous work (4,5), suggests that the nature of the exercise challenge, or the degree of nutritional ketosis are key determinants of the metabolic effects of exogenous ketone supplements during exercise. Although plasma βHB concentrations were elevated to approximately 1.3 mM at the cessation of the 10-km TT, concentrations were approximately 1.2 mM lower than those observed in studies demonstrating effects on plasma glucose and lactate during exercise (4,5). The lower plasma βHB concentrations are a consequence of the aforementioned particulars of the dosing and feeding strategy, and future research should be cognizant of these issues when designing study protocols.

The brain is the primary site of KB utilization under conditions of low carbohydrate availability (30). Elevated βHB concentrations are associated with a neuroprotective role in nonexercise contexts (31–33), and short-term (5 d) feeding of a diet supplemented with KME improved performance of rats in a radial maze task by 38%, and improved decision-making during the test (34). Moreover, in our previous work, acute ingestion of KME preserved cognitive performance, measured by the number of incorrect responses to a multitasking test (4). This test was performed at the cessation of a short duration intermittent run to exhaustion proceeding the Loughborough Intermittent Shuttle Test, a variable intensity running protocol that mimics soccer match-play (35). In contrast to previous results, we observed no difference in cognitive performance with the addition of KME in the present study. The specifics of the exercise challenge may play a role in these divergent findings. The Loughborough Intermittent Shuttle Test is a cognitively demanding task that requires participants to be aware of current and subsequent running speeds for 75 min. Mental fatigue has a negative impact on aspects of cognitive performance, including altered attentional focus (36), and slower and less accurate reaction times (37), suggesting that the more cognitively demanding the task, the larger a deficit in cognitive performance should be evident. In the present study, we observed no decline in cognitive performance in either condition. The absence of decline is important to note because in our previous work, it was a preservation of cognitive performance observed with KME, not an absolute improvement (4). These results suggest the exercise challenge presently employed was not sufficiently cognitively demanding to negatively impact reaction time or executive function, and therefore, potential benefits were unlikely to be observed.

Concerns have been raised about the practical use of exogenous ketone supplements by athletes due to the high rates of occurrence of GI distress in previous work using BD (26), KS (7,17), KDE (12), and KME (4). However, in the present study, incidences of GI distress were similar between conditions, and this is consistent with previous work using KME (5). Typically, rates of occurrence of GI distress are higher with exogenous ketones than with ingestion of water or carbohydrate alone, and GI distress occurs at a higher rate with increasing doses of exogenous ketones (4,7,38). Importantly, no participants nominated GI distress as a distraction or detriment to performance during CHO + KME trials.

The present study has attempted to incorporate several elements of experimental design that are consistent with reviews of best practice when undertaking studies of nutrition supplements and sports performance (24,39–41). These include recruitment of trained participants who compete in the chosen mode of exercise, the inclusion of a familiarization trial to improve the reliability of the performance TT, standardization of

nutrient intakes before and during each trial, the inclusion of an appropriate placebo coupled to the interrogation of the success of the blinding, and a fueling strategy that mimics real-world practice, that is, exercise undertaken in fed conditions rather than fasted, and supported by optimal carbohydrate provision during performance. However, the study is not without limitations. Although the sample size calculations suggested a small sample would be sufficient to detect meaningful change using this experimental protocol, an *n*-size of eight participants is underpowered to explore relationships between performance differences and inter-individual differences in $\dot{V}O_{2max}$ and peak plasma βHB concentrations achieved. These are two parameters that we speculate are important determinants of the performance benefits, if any, of exogenous ketone supplements (27). For example, that three out of eight participants had an improvement in 10-km TT performance that was greater than the SWD is suggestive of potential benefits to performance in certain athletes. Another limitation, despite the strength of the experimental protocol as described above, is that the performance measure employed lacks ecological validity, and is not an entirely accurate representation of a real-world performance scenario because of the preload protocol, and use of a motorized treadmill with self-paced adjustments of speed. The requirement for participants to manually change the treadmill speed using console buttons is dependent upon their perception of an ability to run faster or slower, but may not be sufficiently sensitive to detect small differences in performance (42,43).

In conclusion, the addition of a commercially available ketone monoester supplement to a carbohydrate-based fueling strategy before and during exercise did not improve performance in a self-paced, treadmill-based 10-km TT. Ingestion of the ketone monoester attenuated the rise in plasma glucose before exercise, but concentrations were similar between trials thereafter, and no effect on the increase in plasma lactate concentrations during the 10-km TT was observed. Moreover, no differences between trials were observed for a range of physiological responses, and assessments of cognitive performance. Future research should evaluate different dosing strategies and exercise models to elucidate whether a threshold of plasma βHB concentration must be exceeded to exert performance benefits, and in which exercise contexts these benefits, if any, might be realized.

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The authors declare the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation and do not constitute endorsement by the American College of Sports Medicine.

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