TITLE

Acute ingestion of a ketone monoester without co-ingestion of carbohydrate improves running economy in male endurance runners

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RUNNING TITLE

Ketone monoester improves running economy

ABSTRACT WORD COUNT:	246
WORD COUNT:	4942
REFERENCES:	35
TABLES	2
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ABSTRACT

Purpose: Acute ingestion of a ketone monoester, with and without co-ingestion of carbohydrate, was investigated for effects on running economy (RE), time to exhaustion (TTE), and other related indices of endurance running performance. **Methods:** Using a three condition, placebo-controlled, randomized crossover design, eleven male middle- and long-distance runners ran at five submaximal speeds (10 to 14 $km.h^{-1}$) on a motorized treadmill for 8 min each, immediately followed by a ramp test to volitional exhaustion. Participants consumed either a 10% carbohydrate solution (CHO), a 10% carbohydrate solution with 750 $mg.kg^{-1}$ body mass of a (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (CHO+KE), or 750 $mg.kg^{-1}$ body mass of the ketone monoester in flavored water (KE) before (2/3 of the dose) and during (1/3 of the dose) exercise. **Results:** βHB concentration averaged 1.8±0.3 mM and 2.1±0.3 mM during exercise in CHO+KE and KE, respectively. RE was lower at each submaximal running speed (ES=0.48 to 0.98) by an average of 4.1% in KE compared to CHO, but not between CHO+KE and CHO. TTE did not differ between CHO (369±116 s), CHO+KE (342±99 s), or KE (333±106 s) (P=0.093). **Conclusion:** Acute ingestion of a ketone monoester without carbohydrate, but not when co-ingested with carbohydrate, improved RE in middle- and long- distance runners at a range of submaximal running speeds, and did not alter TTE in a short duration ramp test to volitional exhaustion. Further investigation is required to examine if these differences translate into positive performance outcomes over longer durations of exercise.

KEYWORDS: β-hydroxybutyrate; endurance performance; lactate threshold; oxygen cost; time trial;

INTRODUCTION

The ketone bodies (KB) acetoacetate (AcAc) and β-hydroxybutyrate (βHB) are endogenous compounds produced in the liver from amino acid and fatty acid precursors through the process of ketogenesis (1). The formation of KB provides an additional substrate for multiple tissues, including the brain, heart, and skeletal muscle, where they can be readily oxidized in the mitochondria to support ATP resynthesis and energy provision (2). During periods of reduced carbohydrate availability such as those incurred from prolonged fasting or starvation, or in response to a ketogenic diet, the rate of KB production is increased (1, 3). The concentration of KB can also be rapidly elevated through the acute ingestion of an exogenous ketone supplement, independent of a preceding period of carbohydrate restriction (3). This provision of KB through exogenous means has been touted as a potential ergogenic aid, particularly for endurance performance, given the myriad of metabolic effects associated with acute nutritional ketosis (1). These effects however have failed to consistently translate into improvements in exercise performance (1), and recent studies (4–7) have challenged the initial findings of altered substrate utilization and a major contribution of KB to energy provision during exercise after acute ingestion of an exogenous ketone supplement (8).

Running economy (RE) is a term commonly used to represent the oxygen cost, or energy cost, of submaximal, steady-state running (9). Alongside maximal oxygen consumption ($\dot{V}O_{2max}$), and the fractional percentage of $\dot{V}O_{2max}$ that can be sustained over an extended period of time, RE is recognized as one of the primary physiological factors that contribute to successful endurance running performance (10), and is a stronger predictor of performance than $\dot{V}O_{2max}$ among trained endurance athletes (11). RE is responsive to certain nutrition strategies (11). For example, dietary nitrate supplementation can reduce the oxygen cost of exercise (12–14), whereas low carbohydrate, high fat or ketogenic diets can increase the oxygen cost of exercise (15, 16).

A reduction in the oxygen cost of exercise following acute ingestion of a ketone monoester has been postulated (1). Potential mechanisms are a greater Gibbs' free energy ($\Delta G'$) associated with the generation of ATP from the oxidation of KB over glucose, a higher energy yield per carbon unit expressed either as kJ/C_2 or ATP/C_2 for KB compared to glucose, and an associated reduction in the reliance on glucose during endurance exercise (1, 17), A counterpoint to these mechanisms is the greater phosphorylation-to-oxygen ratio (P/O), which represents metabolic efficiency as the ATP produced per oxygen consumed ($ATP.mol^{-1}$ O_2), for glucose compared to KB (1). A limited number of studies have examined changes in the oxygen or energy cost of exercise, expressed as oxygen consumption ($\dot{V}$ O_2), RE, or cycling efficiency, following the acute ingestion of a ketone monoester, with most reporting no difference (15, 18–20). These studies have typically involved the simultaneous ingestion of carbohydrate, but this may blunt the contribution of KB to energy provision and subsequently lessen any potential metabolic effect (1). Recently, a ketone monoester, ingested in the fasted state and without carbohydrate co-ingestion, improved the delta efficiency of trained endurance athletes cycling at 25%, 50% and 75% of their maximal power output (5). Given that this outcome represents a reduction in the energy cost of exercise, acute nutritional ketosis in the absence of carbohydrate co-ingestion may represent a viable strategy to improve RE as an analogous measure in endurance running performance.

The aim of this study was to examine the effect of acute ingestion of a ketone monoester in the form of the (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (R-BD R-βHB KME), with and without co-ingestion of carbohydrate and compared to carbohydrate alone, on RE across a range of submaximal running speeds in a group of trained endurance runners. A secondary aim of this study was to examine the subsequent effect on time to exhaustion (TTE) during a short duration, ramp test.

METHODS

Participants

Eleven trained male middle- and long-distance runners (age, 35.3±7.5 y; height, 1.83±0.05 m; body mass, 74.6±6.4 kg; sum of 8 skinfolds, 72.1±14.2 mm; $\dot{V}O_{2peak}$, 59.4±5.9 $mL.kg.min^{-1}$) provided written informed consent to participate after written and verbal explanation of the study procedures. Ethical approval was obtained from the Dublin City University Research Ethics Committee (2021_18_BE_SSH). The study was conducted in accordance with the Declaration of Helsinki, with the exception of pre-registration. Participants were required to have a background in endurance running, and self-reported to be training at least 4 times per week and/or for at least 4 hours per week for at least the previous 24 months. All participants were classified as Tier 2/Trained or Tier 3/Highly Trained level athletes based on the recently proposed Participant Classification Framework (21).

Experimental design

A three condition, placebo-controlled, randomized cross-over design was used to examine the effect of acute ingestion of a ketone monoester on RE, TTE, and other related indices of endurance running performance. Participants visited the laboratory on four separate occasions over a 21- to 28-d period for a familiarization trial and three main experimental trials. All trials involved the same exercise protocol, consisting of submaximal treadmill running at five different speeds during which RE was assessed, followed by a ramp test to volitional exhaustion to assess TTE. Only the drink composition differed between trials, which was either a carbohydrate solution with flavored placebo (CHO), a carbohydrate solution with the R-BD R-βHB KME (CHO+KE), or a solution containing the R-BD R-βHB KME with flavored water as a carbohydrate placebo (KE). The primary outcome measure was RE at each submaximal running speed. The *a priori* sample size calculation was based on RE data from a previous study by our group comparing CHO and CHO+KE during submaximal running (1 h

~65%$\dot{V}O_{2peak}$) (18), which resulted in a partial eta squared of 0.575. Using this effect size, an α of 0.05 and β of 0.8, analysis using a repeated measures within-between interaction with three conditions and five timepoints would have required n=6 participants (GPower v3.1). To further increase the statistical power, the target sample size was set at n>10. Secondary outcome measures were changes in blood βHB, blood glucose and blood lactate concentration, minute ventilation ($\dot{V}E$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER), heart rate (HR), ratings of perceived exertion (RPE), $\dot{V}O_{2peak}$, and TTE.

Pretrial preparation

Each visit to the laboratory took place on the same day of the week and at the same time of day ±1 h, separated by 7- or 14-d. All exercise tests commenced between 1800 and 2200 h. Pretrial preparation was the same for all trials, with participants asked to abstain from alcohol for 48 h prior, caffeine 5 h prior, and refrain from strenuous exercise training for 24 h prior to each trial. A standardized meal plan was also prescribed to participants to follow for the day before each trial and for the day of the trial. The meal plan provided 30 kcal.kg^{-1} with a macronutrient composition of 60% carbohydrate, 20% protein, and 20% fat. Participants were advised to consume their final meal ~3 h prior to arriving to the laboratory, which consisted of ~8.5 kcal.kg^{-1} with a macronutrient composition of ~1.2 g.kg^{-1} of carbohydrate, 0.6 g.kg^{-1} of protein, and 0.1 g.kg^{-1} of fat. Compliance with these requests for abstinence, and following of the prescribed meal plan was assessed verbally on arrival for each trial. Hydration status was assessed in urine samples that were taken upon arrival to the laboratory to measure urine specific gravity (USG) using a digital handheld refractometer (PEN-Urine S. G.; Atago Co. Ltd., Tokyo, Japan). Participants were instructed to continue with their normal, unrestricted dietary routine and training program (which included both running and cycling) outside of this

window but were required to record and provide the activity data from their GPS enabled smart watches (Garmin, Olathe, KS USA) for the duration of their involvement.

Anthropometry

Body mass was measured to the nearest 0.1 kg using a calibrated digital scale (model 704; SECA, Hamburg, Germany). Height was measured to the nearest 0.1 cm using a wallmounted stadiometer (model 200; SECA, Hamburg, Germany). Subcutaneous fat tissue was measured to the nearest 0.1 mm by an International Society for the Advancement of Kinanthropometry certified practitioner from 8 anatomical locations (triceps, subscapular, biceps, iliac crest, supraspinale, abdomen, front thigh, and medial calf) using a Harpenden skinfold caliper (Baty International, West Sussex, UK).

Exercise testing

All trials involved a multi-stage incremental running protocol performed on a motorized treadmill (T170; COSMED, Rome, Italy). The first five stages were each 8 min in duration and interspersed with a 30 s rest interval to facilitate the collection of capillary blood samples from the earlobe for determination of blood βHB (FreeStyle Precision Neo; Abbott Industries, Chicago, IL USA), blood glucose (FreeStyle Optium Neo; Abbott Industries, Chicago, IL USA), and blood lactate concentration (Lactate Pro 2; Arkray, Kyoto, Japan). Capillary blood samples were also taken prior to the first bolus of the drink for the respective condition, immediately prior to the start of exercise, and immediately upon cessation of exercise. To allow participants to ingest the second bolus of the drink for the respective condition, the rest interval was increased to 2 min between the second and third stage. Exercise began at 10 $km.h^{-1}$ at a 1% gradient and increased in increments of 1 $km.h^{-1}$ for each stage. Following the fifth stage, the ramp test to volitional exhaustion commenced during which stage durations were reduced to 1 min and running speed was increased by 1 $km.h^{-1}$ every minute up to 16 $km.h^{-1}$,

after which treadmill gradient was increased by 1% until volitional exhaustion. RPE (Borg 6 to 20 scale) was recorded during the last 30 s of each stage.

Expired air was collected and analyzed throughout the entire test using an automated breath-by-breath (BxB) system (Quark RMR; COSMED, Rome, Italy), which was calibrated before each test according to manufacturer recommendations. Participants were fitted with a face mask (7450 V2 Mask; Hans Rudolph, Inc., Shawnee, KS USA) and HR monitor (H10; Polar, Kempele, Finland), and wore the same running shoes in all trials. No smartwatches or activity monitors were permitted during the exercise tests in order to keep participants blinded to their performance. RE, $\dot{V}$ E, $\dot{V}$ O_2, $\dot{V}$ CO_2, and RER were calculated from a 60 s rolling average of BxB measurements during the last 90 s of each 8 min stage (10 to 14 $km.h^{-1}$ inclusive). HR was also calculated from a 60 s rolling average of measurements during the last 90 s. RE is expressed as the volume of oxygen required to run 1 km relative to body mass ($mL.kg^{-1}.km^{-1}$). Short-term, intraindividual variation in RE was calculated using the difference at each submaximal running speed between the familiarization trial and the main CHO experimental trial. The coefficient of variation (CV) was 2.7±1.6%, 1.8±1.3%, 2.6±1.9%, 2.5±2.0%, and 3.5±2.2% at 10 $km.h^{-1}$, 11 $km.h^{-1}$, 12 $km.h^{-1}$, 13 $km.h^{-1}$, and 14 $km.h^{-1}$, respectively. $\dot{V}$ O_{2peak} was recorded as the highest 20 s average observed during the test. The total time (s) completed during the ramp test to volitional exhaustion was recorded as the TTE.

Drink conditions for experimental trials

The protocol for the familiarization trial and main experimental trials were identical except for the drink consumed. Participants ingested the first bolus (500 mL) of a given drink 20 min before commencing the exercise test (-20 min). The second drink (300 mL) was ingested immediately after the 11 $km.h^{-1}$ running stage during the 2 min rest interval. For all participants, the first (familiarization) and fourth trials were CHO, with the order of CHO+KE

and KE randomized between the second and third trial. All drinks were provided in opaque bottles.

CHO consisted of a 10% carbohydrate solution with maltodextrin (100% Maltodextrin Carbs; Myprotein, UK) as the carbohydrate source, and flavored with a lemon sweetener (FlavDrops™; Myprotein, UK). The quantity of carbohydrate ingested was provided to approximate a rate of 1.0 $g.min^{-1}$ of exercise. The R-BD R-βHB KME was in the form of HVMN™ Ketone Ester (HVMN, Inc., San Francisco, CA USA), which was commercially available at the time of the planning of this study, although is no longer available from this source. During CHO, the drink was supplemented with denatonium benzoate, malic acid, and arrow root extract to mimic the bitter taste and mouth-feel of the R-BD R-βHB KME.

During CHO+KE, CHO was supplemented with 750 $mg \cdot kg^{-1}$ body mass of R-BD RβHB KME, for which the ketone monoester was mixed directly with the CHO solution for ingestion. During KE, the 750 $mg \cdot kg^{-1}$ body mass of R-BD R-βHB KME was mixed with water flavored with a lemon sweetener (FlavDrops™; Myprotein, UK) as a carbohydrate placebo. The volume of water ingested in KE was identical to that ingested during CHO and CHO+KE. For each condition, ~2/3 of the total volume was consumed 20 min before commencing exercise, and the remaining ~1/3 consumed between the second (11 $km.h^{-1}$) and third (12 $km.h^{-1}$) stages of the incremental test.

Throughout the testing period, participants were not informed of the exact number of possible combinations of carbohydrate and R-BD R-βHB KME, but were told that they could receive one of the drinks on more than one occasion. After completion of the final experimental trial, participants were asked to identify the trial or trials in which they believed they had received the R-BD R-βHB KME, and the trial in which they believed that they ran for the longest duration during the ramp test to volitional exhaustion.

170 **Gastrointestinal tolerability**

171 Type and severity of gastrointestinal (GI) symptoms were measured using a 12-item
172 questionnaire (22) for each condition prior to the start of exercise, and following completion of
173 the ramp test to volitional exhaustion. Symptom severity was rated on a Likert scale between
174 0 (none) and 8 (unbearable), which were subsequently categorized as none (0), mild (1 or 2),
175 moderate (3 or 4), and severe (5+). Total symptom load at pre and peak exercise was calculated
176 as the sum of participant scores for all symptoms as previously described (22).

177 **Statistical analysis**

178 Statistical analysis was performed using GraphPad Prism v.9.4 (GraphPad Software
179 Inc, CA USA). Data are presented as mean±standard deviation (SD) unless otherwise stated.
180 Body mass, hydration status, weekly training sessions, weekly distance, average heart rate
181 (AHR) and maximum heart rate (MHR) during training were compared between conditions
182 using a one-way repeated measures analysis of variance (ANOVA). A two-way
183 (time*condition) repeated measures ANOVA was performed to determine differences in blood
184 βHB, blood glucose, and blood lactate concentration (time levels: -20 min, 0 min, 10 $km.h^{-1}$,
185 11 $km.h^{-1}$, 12 $km.h^{-1}$, 13 $km.h^{-1}$, 14 $km.h^{-1}$, and peak exercise), RE, $\dot{V}$ E, $\dot{V}$ O_2, $\dot{V}$ CO_2, RER,
186 HR, and RPE (time levels: 10 $km.h^{-1}$, 11 $km.h^{-1}$, 12 $km.h^{-1}$, 13 $km.h^{-1}$, and 14 $km.h^{-1}$). When a
187 main effect of condition or a time*condition interaction effect was indicated, post-hoc testing
188 was performed with Tukey's correction. Multiplicity-adjusted P values are reported to compare
189 CHO, CHO+KE, and KE at the respective time points. A one-way repeated measures ANOVA
190 was used to compare $\dot{V}$ O_{2peak}, HR_{peak}, and TTE between conditions. Sphericity was not
191 assumed, and the Greenhouse-Geiser correction was applied to all ANOVA analyses.
192 Standardized estimates of effect size (ES) were calculated using the ratio of the mean difference
193 to the pooled SD for all primary and secondary outcome variables and are presented as the

194 adjusted Hedges' *g*. Differences between conditions in GI symptoms were examined using
195 Friedman's test with Dunn's post-hoc correction. The significance level was set at $\alpha \leq 0.05$ for
196 all tests.

197

198 **RESULTS**

199 **Body mass, hydration status and training log**

200 Body mass did not differ (P=0.243, ES=0.02 to 0.04) between CHO (74.4±6.2 kg),
201 CHO+KE (74.6±6.5 kg) or KE (74.3±6.4 kg). There was also no difference in hydration status
202 (P=0.705, ES=0.00 to 0.17) between CHO (1.010±0.006 $g.mL^{-1}$), CHO+KE (1.010±0.006
203 $g.mL^{-1}$) or KE (1.009±0.005 $g.mL^{-1}$). The number of training sessions completed each week
204 during the study period was 4.6±2.6. Weekly mileage was 73.4±54.7 km. The AHR and MHR
205 recorded during these sessions was 142±10 bpm and 163±14 bpm, respectively. There was no
206 difference between conditions in the number of training sessions (P=0.303, ES=0.00 to 0.08),
207 weekly distance (P=0.456, ES=0.07 to 0.16), AHR (P=0.665, ES=0.09 to 0.18), or MHR
208 (P=0.799, ES=0.04 to 0.13) in the seven days prior to each trial. No trial order effect was found
209 for number of training sessions (P=0.321), weekly distance (P=0.880), AHR (P=0.903), or
210 MHR (P=0.659).

211 **Blood βHB, glucose and lactate concentration**

212 A time*condition interaction effect (P<0.001) was found for blood βHB concentration
213 (Figure 1A). At 0 min, blood βHB concentration in KE was 2.6±0.6 mM, which was higher
214 than the 2.2±0.8 mM in CHO+KE (P=0.044, ES=0.54). A time*condition interaction effect
215 (P<0.001) was found for blood glucose concentration (Figure 1B). A main effect of time
216 (P<0.001) was found for blood lactate concentration but there was no main effect of condition
217 (P=0.104) or time*condition interaction effect (P=0.129) (Figure 1C).

Submaximal exercise

Main effects of time ($P<0.001$) and condition ($P=0.006$) were found for RE but there was no time*condition interaction effect ($P=0.933$) (Figure 2). RE was lower at 10 $km.h^{-1}$ (mean difference of 7.8, 95% confidence interval (CI)=2.1 to 13.4 $mL.kg.km^{-1}$, $P=0.010$, ES=0.48), 11 $km.h^{-1}$ (mean difference of 8.0, 95% CI=5.3 to 10.7 $mL.kg.km^{-1}$, $P<0.001$, ES=0.71), 12 $km.h^{-1}$ (mean difference of 9.0, 95% CI=1.1 to 16.8 $mL.kg.km^{-1}$, $P=0.026$, ES=0.87), 13 $km.h^{-1}$ (mean difference of 8.4, 95% CI=2.4 to 14.3 $mL.kg.km^{-1}$, $P=0.008$, ES=0.83), and 14 $km.h^{-1}$ (mean difference of 9.1, 95% CI=3.8 to 14.4 $mL.kg.km^{-1}$, $P=0.002$, ES=0.98) during KE compared to CHO (Figure 3A-E). No differences in RE were found between CHO and CHO+KE (all P values for pairwise comparisons >0.05, ES=0.23 to 0.49) or CHO+KE and KE (all P values for pairwise comparisons >0.05, ES=0.31 to 0.54).

Main effects of time ($P<0.001$) were found for $\dot{V}$ E, $\dot{V}$ O_2, and $\dot{V}$ CO_2 (Figure 4A-C), with successive increases between running speeds in all three conditions. A main effect of condition was also found for $\dot{V}$ O_2 ($P=0.004$), and $\dot{V}$ CO_2 ($P=0.003$). A main effect of time ($P<0.001$) and condition ($P=0.024$), and a time*condition interaction effect ($P=0.035$) was found for RER (Figure 4D). During CHO, RER was higher at 10 $km.h^{-1}$ ($P=0.031$, ES=1.00) and 11 $km.h^{-1}$ ($P=0.037$, ES=1.01) compared to KE.

Main effects of time ($P<0.001$) and condition ($P=0.005$) were found for relative $\dot{V}$ O_2, but when expressed as a percentage of $\dot{V}$ O_{2peak} achieved during each respective condition, no main effect of condition ($P=0.991$) was found (Table 1). Main effects of time ($P<0.001$) and group ($P<0.05$) were found for HR and $\%HR_{peak}$ (Table 1). A main effect of time ($P<0.001$) and a time*condition interaction effect ($P=0.015$) was found for RPE. RPE increased at each successive running speed in all conditions (Figure 5).

241 **Ramp test to volitional exhaustion**

242 $\dot{V}O_{2peak}$, expressed in relative terms, was not different between CHO (59.4±5.9
243 mL.kg.min^{-1}) and CHO+KE (58.3±4.8 mL.kg.min^{-1}; P=0.408, ES=0.20), CHO and KE
244 (56.9±5.0 mL.kg.min^{-1}; P=0.080, ES=0.43), or CHO+KE and KE (P=0.187, ES=0.25) (Figure
245 6A). HR_{peak} was lower in KE (180±12 bpm) than CHO (183±13 bpm; P=0.034, ES=0.24) and
246 CHO+KE (182±12 bpm; P=0.032, ES=0.20) (Figure 6B). No difference was found in TTE
247 between CHO (369±116 s) and CHO+KE (342±99 s; P=0.162, ES=0.24), CHO and KE
248 (333±106 s; P=0.187, ES=0.32), or CHO+KE and KE (P=0.803, ES=0.04) (Figure 6C).

249 **Gastrointestinal tolerability**

250 The prevalence of GI symptoms, recorded 5 min prior to the start of exercise and 5 min
251 after the cessation of exercise, for all conditions is presented in Table 2. Total symptom load
252 at pre-exercise was 4 in CHO, CHO+KE and KE. At peak exercise, total symptom load was
253 47, 46, and 69 in CHO, CHO+KE, and KE, respectively. Symptom load did not differ between
254 conditions at peak exercise (P=0.594). During KE, three participants verbally noted GI distress
255 (upper GI, n=1; lower GI, n=2) as limiting their exercise performance during the ramp test to
256 volitional exhaustion.

257 **Blinding**

258 Four (36%) participants correctly identified the condition in which they ran for the
259 longest during the ramp test to volitional exhaustion. Four (36%) participants correctly
260 identified the two trials in which they received the R-BD R-βHB KME, which they attributed
261 to taste alone. Five (45%) participants correctly identified one trial in which they received the
262 R-BD R-βHB KME, while two (18%) participants could not differentiate between trials or
263 chose incorrectly. Based on any factor other than taste, two (18%) participants correctly
264 identified both trials where they received the R-BD R-βHB KME, six (55%) participants

correctly identified one trial, while three (27%) participants could not differentiate between trials or chose incorrectly.

DISCUSSION

This study investigating the effect of acute ingestion of the R-BD R-βHB KME on RE in a cohort of trained male middle- and long-distance runners found that in KE, but not CHO+KE, RE was improved by 3.6% to 4.5% at each of the five submaximal running speeds compared to CHO. These changes occurred in conjunction with a reduction in $\dot{V}O_2$ and $\dot{V}CO_2$ at each submaximal running speed. TTE, $\dot{V}O_{2peak}$, and blood lactate concentrations were similar across all three conditions.

The oxygen or energy cost of steady-state running at a given speed, commonly referred to as RE, is recognized as one of the most important physiological factors influencing longdistance running performance (9, 23, 24). Both short- and long-term training, and nutrition interventions have targeted changes in RE with mixed results reported (11, 12, 16, 25). The development and commercialization of exogenous ketone supplements in the last decade has presented a viable strategy by which RE may be improved. This is due to the range of modulatory effects that KB exert on metabolism during exercise, including alterations in substrate utilization, reduced hepatic glucose output, and a lower concentration of free fatty acids that may subsequently reduce the energy cost of exercise (1).

In a related study of the effect of acute ingestion of the R-BD R-βHB KME, involving trained endurance athletes cycling at 25%, 50%, and 75% of their maximal power output, acute ingestion of 252 $mg.kg^{-1}$ body mass of R-BD R-βHB KME resulted in an ~7% improvement in delta efficiency compared to a fasted, calorie-free control (5). Exercise efficiency in cycling is analogous to RE by representing a quotient of the change in energy expenditure and actual

work completed. This ~7% improvement was attributed to small, albeit not statistically significant, reductions in $\dot{V}O_2$ and $\dot{V}CO_2$, with whole blood βHB concentration averaging ~1.8 mM across the three exercise intensities (5). A 'high dose' condition was also included in the latter study providing 752 $mg.kg^{-1}$ body mass of R-BD R-βHB KME during which βHB concentration averaged ~4.2 mM, but delta efficiency in this condition did not differ from the low dose or control (5). The absence of effect in the high dose condition may indicate that over a certain threshold of βHB concentration there is no dose-response for the effects of ingestion of R-BD R-βHB KME on metabolism during exercise, or the small sample size (n=6) may have simply resulted in an underpowered analysis. In the present study, resting whole blood concentrations of βHB were elevated to 2.2 mM and 2.6 mM during CHO+KE and KE, respectively. Concentrations of βHB declined following the first two submaximal running stages, but rose steadily after consumption of the second bolus, reaching average concentrations of 2.2 mM and 2.4 mM at peak exercise in CHO+KE and KE, respectively.

The absence of carbohydrate being co-ingested with R-BD R-βHB KME in the abovementioned study (5) may have contributed to the effect observed, because co-ingestion of carbohydrate may reduce the oxidation of KB, and consequently the potential effects of acute nutritional ketosis on metabolic responses to exercise (1). This mechanism has been demonstrated *in vitro* where the addition of pyruvate to KB-supported respiration in isolated skeletal muscle mitochondria increased respiration, whereas KB had a negligible effect on pyruvate-supported respiration (26). This observation may inform the findings of the present study in which improvements in RE were found at each submaximal running speed in KE, but not CHO+KE, despite participants receiving the same dose of R-BD R-βHB KME in each condition.

A directional, albeit not statistically significant, improvement in RE was evident in

CHO+KE, with the average across the five submaximal running speeds being ~1.9%. A similar reduction of ~2.6%, although again not statistically significant, was found in our previous work during 60 min of treadmill-based running at ~65%$\dot{V}O_{2max}$ where trained runners consumed CHO+KE containing 573 $mg.kg^{-1}$ body mass of the same R-BD R-βHB KME (18). That observation and the improvement in delta efficiency without the co-ingestion of carbohydrate (5) were the major impetuses for the present study. Collectively, these studies are supportive of the potential inhibitory effect of other substrates on KB oxidation and/or related metabolic effects of acute nutritional ketosis. For example, attenuations in the rise in blood lactate concentrations during exercise are often used as an indirect marker of changes in substrate utilization indicating reduced reliance on carbohydrate as a substrate, and have been observed previously in response to acute ingestion of R-BD R-βHB KME (8, 27–29). The absence of differences in blood lactate concentrations in the present study is likely a consequence of the submaximal running stages being at speeds that are below, or slightly above, the lactate threshold. Moreover, several investigations have reported no differences in RE, $\dot{V}O_2$, or related variables after acute ingestion of exogenous ketone supplements (15, 19, 20, 30), but methodological differences including the absence of a condition providing R-BD R-βHB KME alone (15, 19, 20), use of ketone salts (30), cycling rather than running (19, 20, 30), and absence of a control group (15) limit direct comparison.

The influence of GI distress in the present study should also be considered. Three participants verbally reported that GI distress had a negative impact on performance during the ramp test to volitional exhaustion in KE, yet the overall dataset did not reveal differences between the three conditions. The cause of GI distress with the acute ingestion of exogenous ketone supplements is unclear, and highly variable, dependent on the type and dose of exogenous ketone supplements, prior food intake, nutrients co-ingested, and the nature of the exercise challenge (1).

The smallest worthwhile change of practical significance in RE at 14 km.h^{-1} has been reported to be 2.6% for highly trained distance runners (24). In comparison, RE at 14 km.h^{-1} was only lower by 1.9% in CHO+KE compared to CHO, whereas during KE, RE was 4.5% lower compared to CHO. Moreover, the test-retest reliability score of RE in the present study expressed as a CV was ~1.8 to 3.5%, i.e. also lower than the average improvement in RE during KE, which is central to the interpretation of the smallest worthwhile change (31). To further reduce the potential influence of external factors on RE, dietary intake, training activities, the laboratory environment, the number of days between trials, the time of day, and the footwear used, were all standardized across the four trials as previously recommended (9). Despite these strengths, the positive results of the present study in relation to KE are preliminary inasmuch as it is not possible to conclude if this effect will translate into a practically-meaningful change in middle-, long- or ultra-distance running performance. Investigations incorporating acute ingestion of R-BD R-βHB KME compared with or without co-ingestion of carbohydrate over running distances and durations similar to those in long distance events are now required. Notably, the changes in RE are similar to those elicited from technological advances in footwear (32), which have subsequently contributed to an improvement in elite endurance running performance (33).

The decision to limit the inclusion criteria to male athletes is a limitation of the present study. Given the established between-sex differences in substrate utilization during submaximal, long duration running (34), it is possible that there are between-sex differences in the effects of acute nutritional ketosis on the metabolic response to exercise, rates of KB oxidation, and/or any related changes in RE. These questions are unexplored at present and will require separate studies. RE can be presented as energy cost (kcal.kg^{-1}.km^{-1}), which is calculated from the $\dot{V}O_2$ and RER at a given exercise intensity, and this may be a more appropriate measure than mL.kg.km^{-1} as the former accounts for differences in substrate

363 utilization (23). However, this approach is not suitable under conditions of ketosis, or for
364 measuring changes in rates of KB oxidation from indirect calorimetry, given the similar RER
365 of βHB (0.89) and AcAc (1.0) to carbohydrate oxidation (1, 35).

366 In addition to the inappropriateness of indirect calorimetry to assess changes in
367 substrate utilization under conditions of nutritional ketosis (35), it was not possible to measure
368 the uptake and oxidation of KB into skeletal muscle or other tissues such as the heart to
369 determine the full extent of acute nutritional ketosis on exercise metabolism. The current
370 understanding is that the contribution of KB to overall energy expenditure during exercise is
371 less than 5% after the acute ingestion of R-BD R-βHB KME (5), and acute nutritional ketosis
372 has somewhat minor effects on substrate utilization during exercise (4–7). Therefore, the
373 incorporation of skeletal muscle biopsies, inclusion of additional blood markers such as insulin
374 and FFAs, and isotope tracers to isolate the contribution of the various substrates, are required
375 if future studies are to provide additional mechanistic insights.

376 In summary, acute ingestion of a R-BD R-βHB KME resulted in an improvement in RE
377 across a range of submaximal running speeds in a cohort of trained, male middle- and
378 longdistance runners, when consumed alone but not when co-ingested with CHO. TTE was
379 not different during a ramp test to volitional exhaustion between the different conditions. An
380 improvement in RE potentially represents a change in the metabolic response to exercise that
381 may subsequently translate into an improvement in endurance running performance. Future
382 research should, therefore, investigate the effect of acute ingestion of R-BD R-βHB KME (or
383 exogenous ketone supplements producing similar changes in circulating βHB concentrations)
384 without co-ingestion of carbohydrate on endurance performance over exercise durations of >2
385 h with male and female endurance runners.

386

ACKNOWLEDGEMENTS

The authors thank Dr. Katherine Veras and Mr. Scott Burleigh for technical assistance during the exercise testing, and Mr. Mark Germaine for performing the skinfold measurements. No conflict of interest, financial or otherwise, is declared by the authors.

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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TABLES

Table 1. Relative $\dot{V}O_2$ and HR at each submaximal running speed during CHO, CHO+KE and KE.

	Condition	10 km.h^{-1}	11 km.h^{-1}	12 km.h^{-1}	13 km.h^{-1}	14 km.h^{-1}	P (time)	P (condition)	P (interaction)
$\dot{V}O_2$ (mL.kg.min^{-1})	CHO	36.1 ± 2.7	38.1 ± 2.1	40.8 ± 1.9	44.0 ± 1.7	47.2 ± 2.0	<0.001	0.005	0.649
	CHO+KE	35.5 ± 2.0	37.4 ± 2.3	39.8 ± 2.1	43.2 ± 2.2	46.3 ± 2.2			
	KE	34.8 ± 2.5**	36.6 ± 1.9***	39.0 ± 2.1*	42.2 ± 2.4**	45.1 ± 2.1**			
$\%\dot{V}O_{2peak}$	CHO	61.3 ± 7.4	64.6 ± 7.2	69.3 ± 7.0	74.8 ± 7.6	80.1 ± 8.0	<0.001	0.991	0.929
	CHO+KE	61.4 ± 6.8	64.6 ± 7.3	68.8 ± 7.3	74.7 ± 7.7	80.0 ± 8.1			
	KE	61.5 ± 6.5	64.7 ± 6.5	69.1 ± 8.4	74.7 ± 8.5	79.8 ± 9.0			
HR (bpm)	CHO	126 ± 11	134 ± 12	143 ± 12	153 ± 11	162 ± 11	<0.001	0.040	0.103
	CHO+KE	130 ± 10	138 ± 10*	146 ± 10	155 ± 10	163 ± 11			
	KE	127 ± 8‡	136 ± 8	143 ± 9‡	152 ± 9‡	160 ± 10‡‡			
$\%HR_{peak}$	CHO	68.9 ± 4.3	73.5 ± 4.4	78.3 ± 4.7	83.9 ± 4.2	88.6 ± 4.3	<0.001	0.021	0.166
	CHO+KE	71.2 ± 3.1*	75.8 ± 2.5*	80.0 ± 3.2	85.1 ± 3.1	89.6 ± 3.5			
	KE	71.0 ± 2.4	76.1 ± 2.4	79.6 ± 4.2	84.9 ± 4.2	89.4 ± 4.3			

Data are presented as mean ± SD. $\dot{V}O_2$, oxygen consumption; HR, heart rate. * P<0.05 vs CHO; ** P<0.01 vs CHO; *** P<0.001 vs CHO; ‡ P<0.05 vs CHO+KE; ‡‡ P<0.01 vs CHO+KE.

Table 2. Gastrointestinal (GI) symptoms prior to and at the cessation of exercise in CHO, CHO+KE and KE.

	Pre exercise			Peak exercise		
	CHO	CHO+KE	KE	CHO	CHO+KE	KE
Upper GI						
None	10	10	10	7	3	4
Mild	1	1	1	3	7	5
Moderate	0	0	0	0	1	2
Severe	0	0	0	1	0	0
Total with symptoms	1	1	1	5	8	7
Lower GI						
None	9	11	11	7	7	5
Mild	2	0	0	0	4	3
Moderate	0	0	0	4	0	3
Severe	0	0	0	0	0	0
Total with symptoms	2	0	0	4	4	6
Systemic						
None	10	8	9	6	4	4
Mild	1	3	2	3	5	3
Moderate	0	0	0	2	2	4
Severe	0	0	0	0	0	0
Total with symptoms	1	3	2	5	7	7

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FIGURES

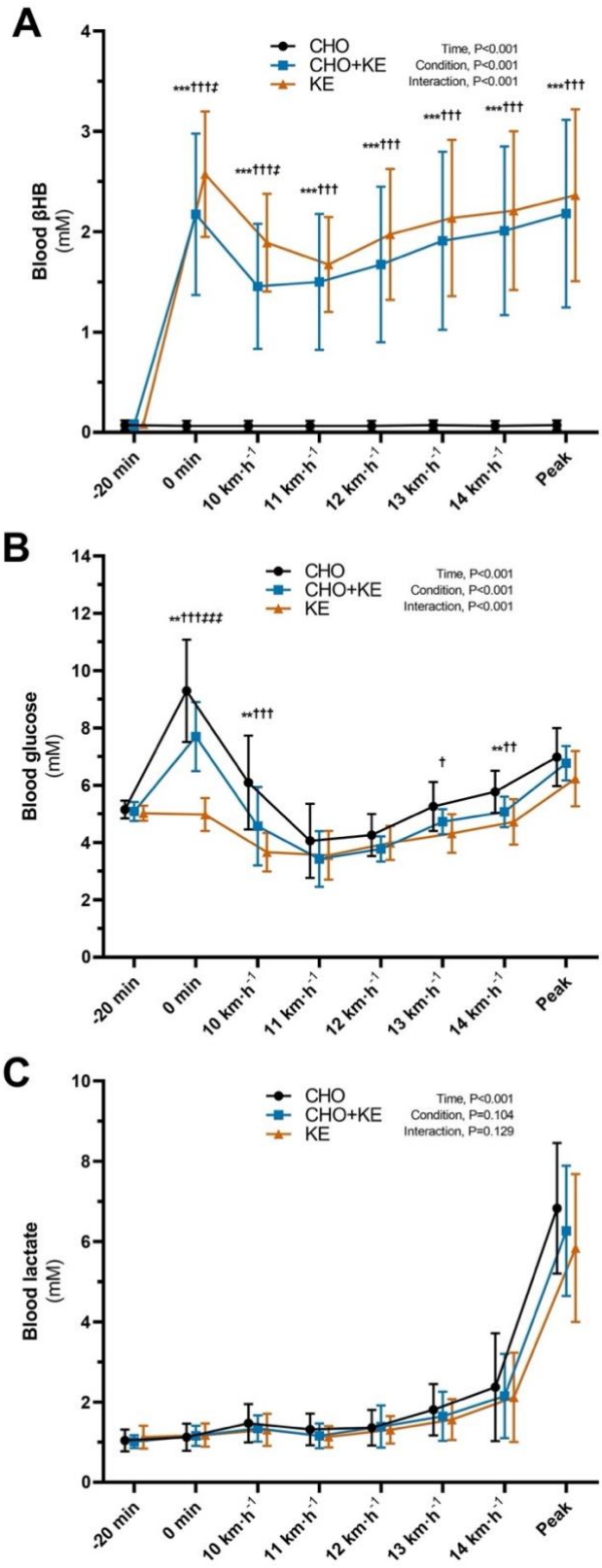

Figure 1. Blood βHB (A), blood glucose (B), and blood lactate (C) concentration during CHO, CHO+KE, and KE. Data are presented as mean values with error bars representing the SD. *** P<0.001 CHO vs

CHO+KE; ** P<0.01 CHO vs CHO+KE; †† P<0.01 CHO vs KE; ††† P<0.001 CHO vs KE; ♯ P<0.05 CHO+KE vs KE; ♯♯♯ P<0.001 CHO+KE vs KE.

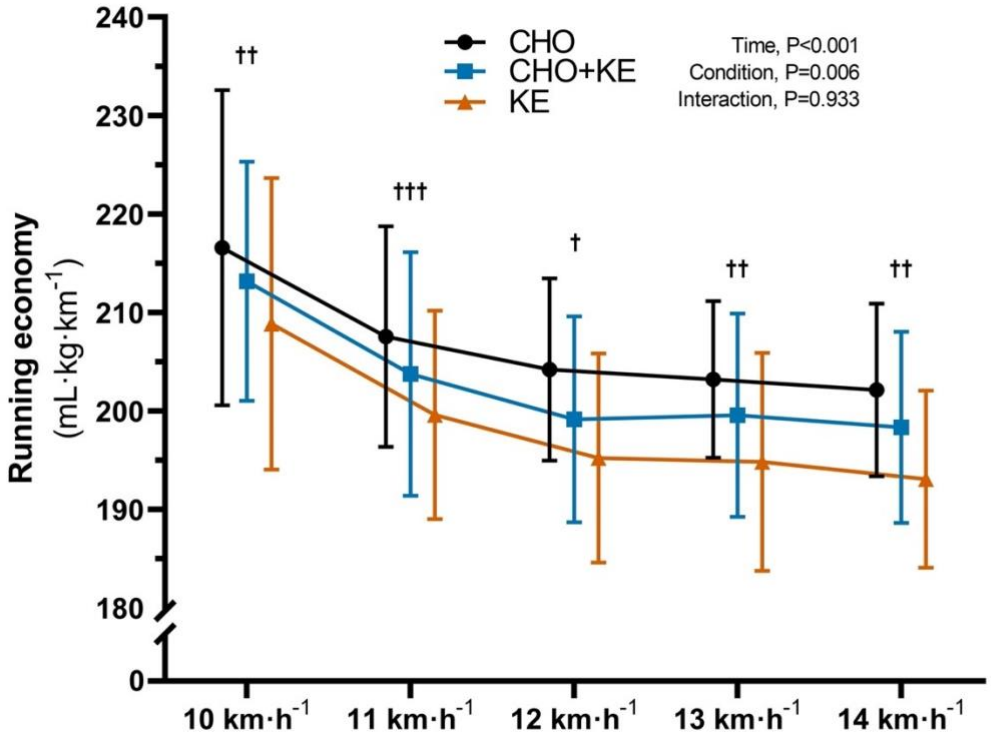

Figure 2. RE at each submaximal running speed during CHO, CHO+KE and KE. Data are presented as mean with error bars representing SD. † P<0.05 CHO vs KE; †† P<0.01 CHO vs KE; ††† P<0.001 CHO vs KE.

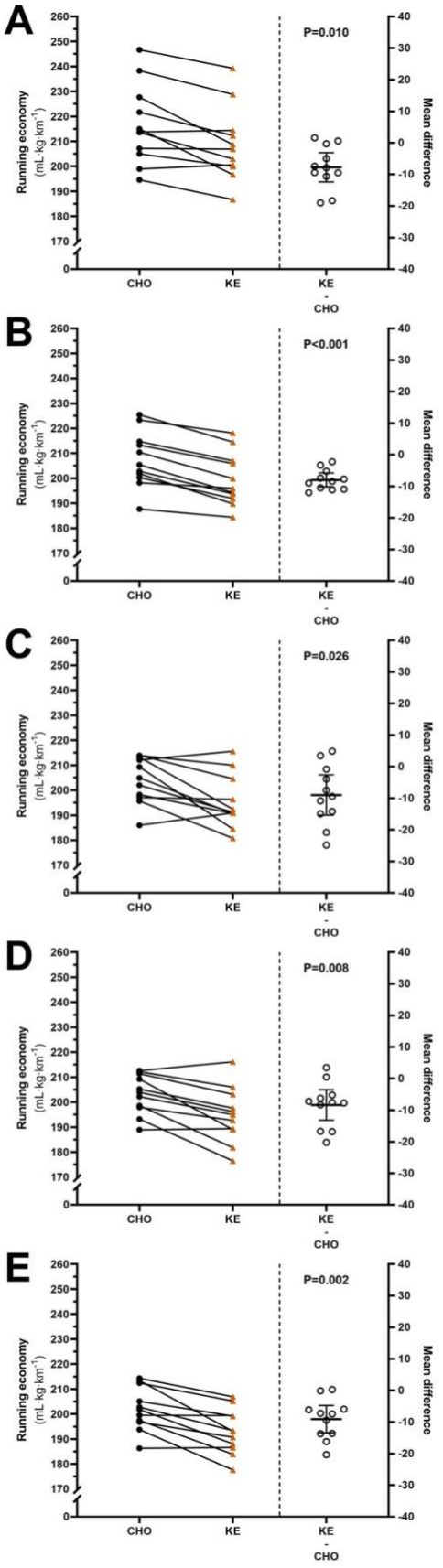

Figure 3. Pairwise comparison of RE between CHO and KE and the mean difference at 10 km.h^{-1} (A), 11 km.h^{-1} (B), 12 km.h^{-1} (C), 13 km.h^{-1} (D), and 14 km.h^{-1} (E). Filled circles and traingles represent the individual

data points from CHO and KE, respectively (left); Clear circles represent the individual difference between conditions with data presented as mean and error bars representing 95% CI (right).

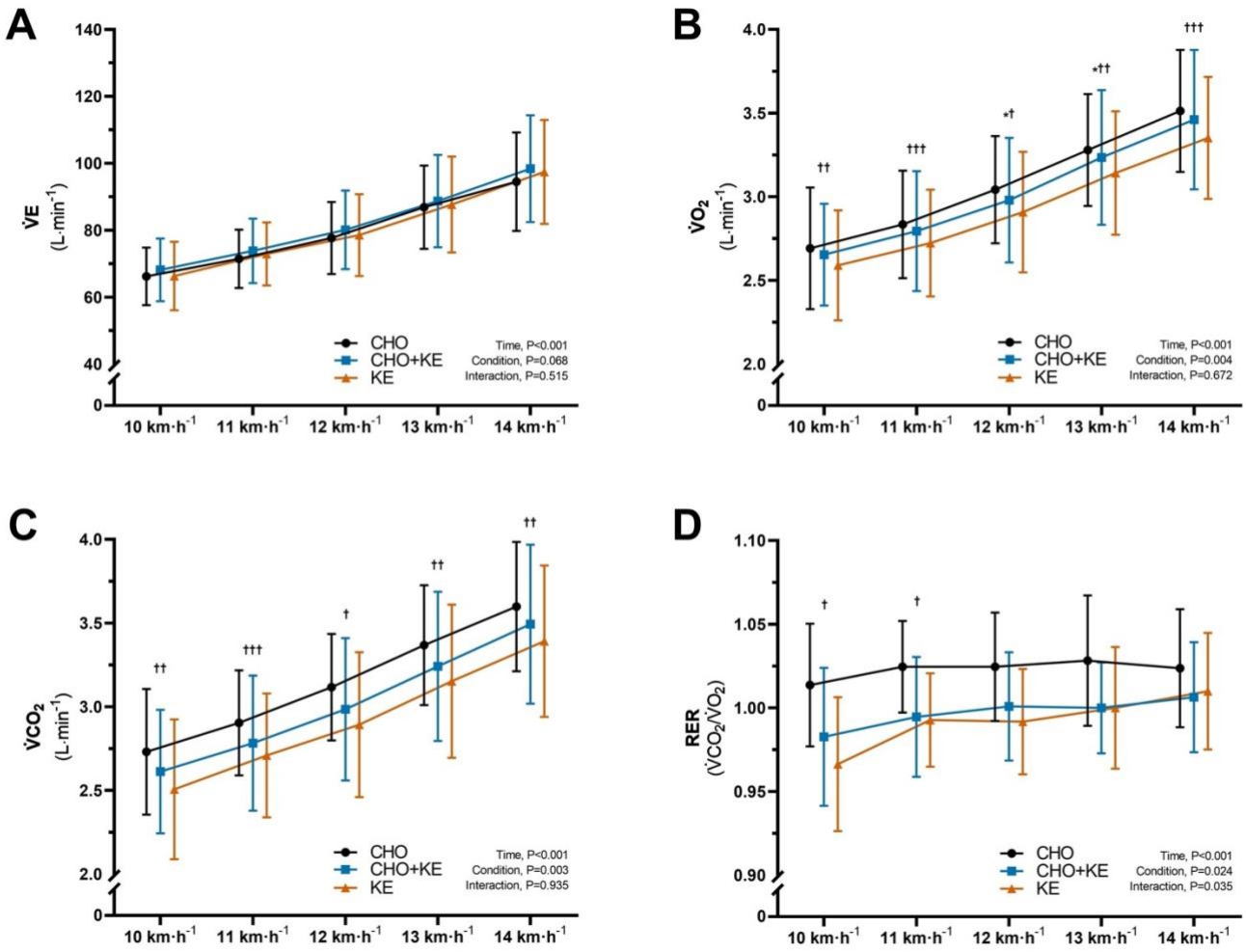

Figure 4. Minute V̇ E (A), V̇ O_2 (B), V̇ CO_2 (C), and RER (C) at each submaximal running speed during CHO, CHO+KE and KE. Data are presented as mean with error bars representing SD. * P<0.05 CHO vs CHO+KE; † P<0.05 CHO vs KE; †† P<0.01 CHO vs KE; ††† P<0.001 CHO vs KE.

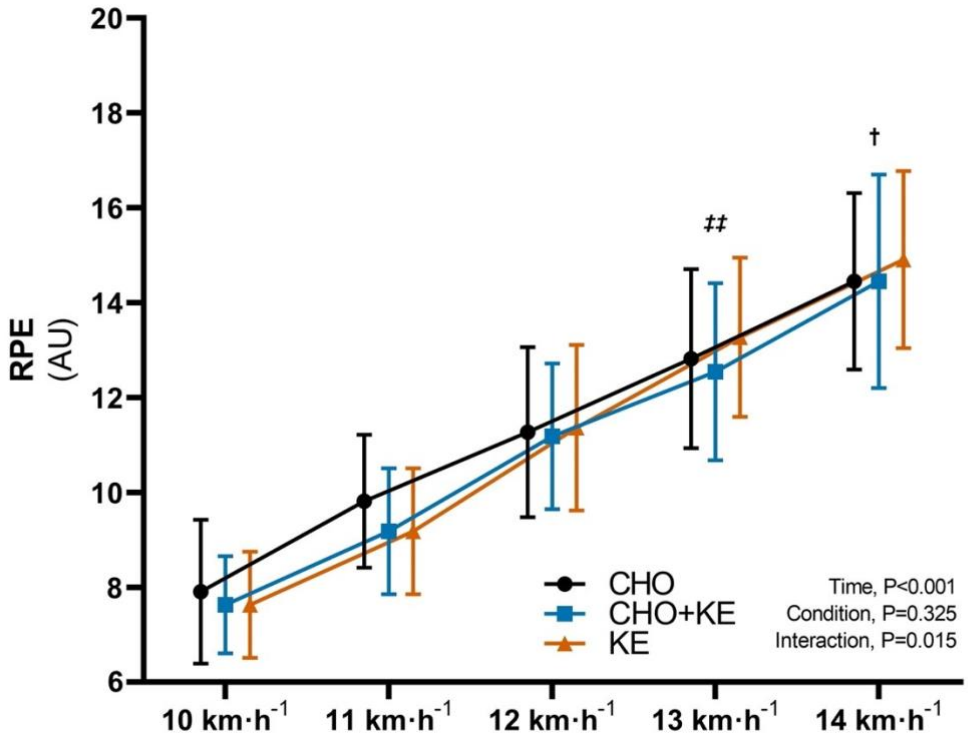

Figure 5. RPE at each submaximal running speed during CHO, CHO+KE and KE. Data are presented as mean with error bars representing SD. † P<0.05 CHO vs KE; ‡‡ P<0.01 CHO+KE vs KE.

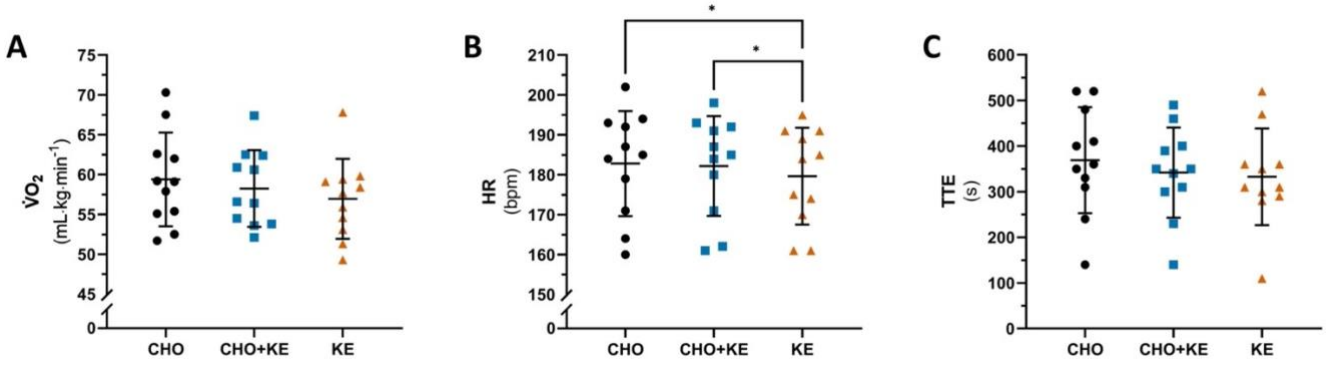

Figure 6. $\dot{V}O_{2peak}$ (A), HR_{peak} (B), and TTE (C) during CHO, CHO+KE and KE. Filled circles, squares and triangles represent the individual data points from CHO, CHO+KE and KE, respectively, with error bars representing SD. * P<0.05.