TITLE
Advanced footwear technology, but not acute ingestion of a ketone monoester, improves running economy in middle- and long-distance runners

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RUNNING TITLE
AFT but not KME improves running economy

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ABSTRACT

Purpose: This study examined the separate and combined effects of advanced footwear technology (AFT) and acute ingestion of a ketone monoester on running economy (RE), time-to-exhaustion (TTE), and other metabolic and cardiorespiratory parameters. **Methods:** In a four-condition, placebo-controlled, randomized crossover design, 18 middle- and long-distance runners (male/female, 10/8, $\dot{V}O_{2peak}$: 59.4±7.2 $mL \cdot kg^{-1} \cdot min^{-1}$) completed five 8 min stages of submaximal running (male: 10–14 $km \cdot h^{-1}$; female: 9–13 $km \cdot h^{-1}$) on a motorized treadmill, immediately followed by a ramp test to volitional exhaustion. Participants consumed 500 mL of either a 10% carbohydrate solution (CHO) or 500 $mg \cdot kg^{-1}$ body mass of an (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester with flavored water (KME) 20 min before exercise, and an additional 300 mL of the 10% carbohydrate solution or 250 $mg \cdot kg^{-1}$ body mass of KME during exercise, while wearing either Nike Pegasus Turbo (PEG) or Nike ZoomX Vaporfly Next% 3 (VAP) running shoes. The four randomized conditions were PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME. **Results:** RE was significantly improved during the third and fourth submaximal running stages in VAP+CHO and VAP+KME compared to PEG+CHO and PEG+KME (all $P<0.05$; ES=0.53-0.84). RE was also improved during the fifth submaximal running stage in VAP+KME compared to PEG+CHO, and in VAP+CHO and VAP+KME compared to PEG+KME (all $P<0.05$; ES=0.56-0.66). No differences in RE were found between CHO and KME conditions. TTE was significantly longer in VAP+CHO (381±125 s) than PEG+CHO (356±140 s; ES=0.18, P=0.023) and PEG+KME (329±131 s, ES=0.40, $P<0.001$) and in VAP+KME (375±125 s) than PEG+KME (ES=0.35, $P<0.001$). **Conclusions:** AFT, but not the acute ingestion of a ketone monoester, improved the RE of trained male and female middle- and long-distance runners at submaximal running speeds.

KEYWORDS: β-hydroxybutyrate; carbon fiber plate; energy cost; ergogenic aid; Vaporfly;

INTRODUCTION

Running economy (RE) refers to the energy or oxygen cost associated with submaximal, steady-state running (1). RE is an important contributor to endurance performance alongside maximal oxygen consumption ($\dot{V}O_{2max}$), and is considered a more accurate predictor of distance running performance among elite endurance athletes than $\dot{V}O_{2max}$ (2). RE can account for ~65% of the variation in 10 km race performance among highly trained middle- and long-distance runners (3) and can differ by as much as 30% between elite endurance athletes (4). A wide range of training, nutrition, and technological interventions have targeted improvements in RE, with the expectation that improvements in RE can result in improvements in middle- and long-distance running performance (2).

Advanced footwear technology (AFT) has gained significant attention as a strategy to improve RE since an AFT prototype demonstrated an average improvement of 4.2% compared to a non-AFT running shoe (5). Running shoes with AFT feature a resilient midsole foam, and a carbon fiber plate (CFP) strategically positioned within the midsole, both designed to optimize energy return and minimize energy expenditure through passive elastic recoil (6–8). The potential performance-enhancing effects of AFT is suggested by the commercialization of these shoe types being associated with improvements in competitive marathon times of, on average, 1.2% and 2.0% for elite male and female athletes, respectively (9). The effect of AFT on RE, particularly the Nike ZoomX Vaporfly (10), has been repeatedly shown as beneficial reductions in oxygen consumption ($\dot{V}O_2$) for both male and female athletes of varying training levels and across a range of running speeds compared to running shoes without AFT (5, 11, 12). However, the reported improvements of ~7% and decrements of ~1% within a single study underscore the extent of inter-individual variation that can exist with AFT (11).

Exogenous ketone supplements (EKS) have also received considerable interest in recent years due to their effects on substrate utilization in skeletal muscle and potential glycogen sparing effects that may benefit endurance performance (13). The ketone bodies (KB) acetoacetate and β-hydroxybutyrate (βHB) are organic compounds synthesized primarily in the liver from fatty acids via ketogenesis, whose concentration can be rapidly elevated after EKS ingestion (13). These KB serve as an additional energy source for various tissues including the brain, heart, and skeletal muscle (14, 15). KB may provide a more efficient source of ATP compared to glucose via a greater Gibbs' free energy (ΔG') and higher

energy yield per carbon unit when expressed as kJ/C_2 or ATP/C_2, which may in turn reduce oxygen cost and/or the reliance on glucose metabolism during prolonged exercise (13). Conversely, the contribution of βHB oxidation to energy provision during exercise is relatively minor and has ranged from ~2.5 to 8.4% (16, 17), whereas the effect of EKS ingestion on exercise performance has been equivocal to date in many studies reporting null effects (13). Yet, our group found an average improvement in RE of 4.1% for male middle- and long-distance runners across five submaximal running speeds (10 to 14 $km \cdot h^{-1}$) following the acute ingestion of the (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (R-BD R-βHB KME) in the fed state without co-ingestion of carbohydrate compared to a carbohydrate control (18). This improvement was not observed when the R-BD R-βHB KME was co-ingested with carbohydrate (18).

AFT and the acute ingestion of the R-BD R-βHB KME are evidently both capable of eliciting improvements in RE of ~4%, and so it is plausible that their combination may work synergistically by having effects on RE through different mechanistic actions i.e., biomechanical and metabolic, respectively. Yet, synergistic effects on performance outcomes are often not observed, at least when different dietary supplements or bioactive compounds are co-ingested as ergogenic aids (19). The primary aim of this study was to investigate if the effect of AFT in combination with the acute ingestion of the R-BD R-βHB KME on the RE of trained male and female middle- and long-distance runners is greater than the effect of AFT or acute ingestion of the R-BD R-βHB KME alone. Stated as a null hypothesis, we hypothesized that the combination of AFT and acute ingestion of the R-BD R-βHB KME would not result in greater changes in RE than those changes resulting from AFT or R-BD R-βHB KME ingestion alone.

METHODS

Participants

Eighteen middle- and long-distance runners (male/female, 10/8; age: 26.0±6.5 years; height: 1.76±0.09 m; body mass: 66.3±9.8 kg; $\dot{V}O_{2peak}$: 59.4±7.2 $mL \cdot kg^{-1} \cdot min^{-1}$) provided written informed consent to participate after written and verbal explanation of the study procedures. Ethical approval for the study was granted from the Dublin City University Research Ethics Committee (permit number: DCUREC/2023/180). The study was conducted in accordance with the Declaration of Helsinki, with the exception of preregistration in a trial database. All participants had a background in middle- or long-distance running and were regularly training at least four times per week and/or for at least four hours per week over the past 24 months. According to the Participant Classification Framework, participants were classified as tier 2/trained (n=11) or tier 3/highly trained (n=7) level athletes (20).

Experimental approach to the problem

A four condition, placebo-controlled, randomized crossover design was used to examine the effect of AFT and acute ingestion of the R-BD R-βHB KME alone, and in combination, on the RE of trained middle- and long-distance runners. Participants completed four separate experimental trials over a 21- to 35-d period. Each trial consisted of the same exercise protocol, involving submaximal treadmill running at five different speeds to examine RE, followed immediately by a ramp test to volitional exhaustion to assess $\dot{V}O_2$peak and TTE (21). The only difference between trials was the drink composition and/or the type of running shoe. The drinks provided were either a carbohydrate solution with a flavored placebo (CHO) or a solution containing the R-BD R-βHB KME mixed with water (KME). The running shoes provided were either the Nike Pegasus Turbo (PEG) or the Nike ZoomX Vaporfly Next% 3 (VAP). The four visits were, therefore, PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME. The primary outcome measure was changes in RE across the range of submaximal running speeds with secondary outcome measures of changes in blood βHB, blood glucose, and blood lactate concentration, minute ventilation ($\dot{V}E$), $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER), heart rate (HR), ratings of perceived exertion (RPE), $\dot{V}O_2$peak, and TTE.

Pretrial preparation

Participants visited the laboratory 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 1100 and 2000 h. Pretrial preparation was the same for all trials, with participants asked to abstain from alcohol for >48 h prior and caffeine for >5 h prior, and refrain from strenuous exercise training for >24 h prior to each trial. Participants were also asked to follow a standardized meal plan on the day prior to and day of each trial. The meal plan provided 30 $kcal \cdot kg^{-1} \cdot day^{-1}$ with a macronutrient composition of 60% carbohydrate, 20% protein, and 20% fat. Food intake on the day of the trial was adjusted proportionally to the time at which the exercise was performed. Participants were advised to consume their last meal ~3 h prior to arrival at the laboratory which consisted of ~8.5 $kcal \cdot kg^{-1}$ with a macronutrient composition of ~1.2 $g \cdot kg^{-1}$ of carbohydrate, 0.6 $g \cdot kg^{-1}$ of protein, and 0.1 $g \cdot kg^{-1}$ of fat. Compliance with these procedures and with the meal plan were confirmed verbally on arrival to the laboratory. Hydration status was assessed from a urine sample collected upon arrival to the laboratory to measure urine specific gravity using a digital handheld refractometer (PEN-Urine S. G.; Atago Co. Ltd., Tokyo, Japan) with body mass measured to the nearest 0.1 kg using a calibrated digital scale (model 704; SECA, Hamburg, Germany) and height measured to the nearest 0.1 cm using a wall-mounted stadiometer (model 200; SECA). Participants were free to continue with their normal, unrestricted dietary routine and training program outside of this window but were required to record and provide the activity data from their GPS-enabled smart watches for the duration of their involvement. During their first visit, female participants were asked to provide information on their menstrual characteristics to assess cycle phase and hormonal contraceptive use. Responses to this questionnaire were not, however, subject to exclusionary criteria given the lack of high-quality evidence demonstrating a significant effect of menstrual cycle phase and hormonal contraceptive use on exercise performance (22, 23), coupled with this study being a crossover design in which participants serve as their own controls. Of the eight female participants, six participants were not using any form of oral or hormonal contraceptive but one of whom was using a intrauterine device (IUD; copper coil), while one participant was using a hormonal contraceptive in the form of a hormonal IUD (Mirena®; Bayer AG, Leverkusen, Germany) and another participant was using an oral contraceptive pill (Azalia®; Gedeon Richter Ireland, Dublin, Ireland).

106

107 *Exercise protocol*

108 All trials involved a multistage incremental running protocol performed on a motorized
109 treadmill (T170; COSMED, Rome, Italy) as previously described (21) and shown in Figure 1. The first
110 five stages were each 8 min in duration and interspersed with a 30 s rest interval to facilitate the
111 collection of capillary blood samples from the earlobe for determination of blood βHB (FreeStyle
112 Precision Neo; Abbott Industries, Chicago, IL), blood glucose (FreeStyle Optium Neo; Abbott
113 Industries), and blood lactate concentration (Lactate Pro 2; Arkray, Kyoto, Japan). Capillary blood
114 samples were also taken prior to consumption of the first bolus of the drink for the respective condition,
115 immediately before the start of exercise, and immediately upon cessation of exercise. The rest interval
116 between the second and third exercise stages was extended to 2 min to allow sufficient time for
117 participants to ingest the second bolus of the drink for the respective condition. For male participants,
118 exercise began at 10 $km \cdot h^{-1}$ with a 1% gradient and increased by 1 $km \cdot h^{-1}$ each stage up to and inclusive
119 of 14 $km \cdot h^{-1}$. For female participants, exercise began at 9 $km \cdot h^{-1}$ with a 1% gradient and increased by
120 1 $km \cdot h^{-1}$ each stage up to and inclusive of 13 $km \cdot h^{-1}$. Throughout the remainder of the text, these five
121 stages will be uniformly referred to as Stage 1, Stage 2, Stage 3, Stage 4, and Stage 5 for both male and
122 female participants. After the fifth stage, stage duration was reduced to 1 min for all participants, and
123 running speed was increased by 1 $km \cdot h^{-1}$ at the end of each of the next two stages, after which the
124 treadmill gradient rose by 1% each minute until volitional exhaustion. RPE (Borg 6 to 20 scale) was
125 recorded during the last 30 s of each stage.

126 Expired air was collected and analyzed throughout the entire exercise test using an automated
127 breath-by-breath system (Quark RMR; COSMED, Rome, Italy). Participants were fitted with a face
128 mask (7450 V2; Hans Rudolph, Inc., Shawnee, KS) and a HR monitor (Polar H10; Kempele, Finland)
129 prior to exercise, and were instructed to remove all activity monitors to ensure they were blinded to
130 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 breath-
131 by-breath measurements during the last 90 s of each 8 min stage. HR was also calculated from a 60 s
132 average of measurements during the last 90 s. $\dot{V}O_{2peak}$ was recorded as the highest 20 s average observed

during the test. The total time in seconds completed during the ramp test to volitional exhaustion was recorded as the TTE. The reliability of RE and related cardiorespiratory measurements arising from this protocol has been reported for male-middle and long-distance runners previously (21). Expressed as a coefficient of variation, the reliability of RE was 2.7%, 2.2%, 2.3%, 2.6%, and 3.1% at 10 $km \cdot h^{-1}$, 11 $km \cdot h^{-1}$, 12 $km \cdot h^{-1}$, 13 $km \cdot h^{-1}$, and 14 $km \cdot h^{-1}$, respectively. The reliability of $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER, HR, and $\dot{V}O_2peak$ was <5% across the five submaximal running speeds, and was 7.2% for TTE (21).

Drink conditions and running shoes for experimental trials

The protocol for the experimental trials was identical except for the drink consumed and the running shoes provided (Figure 1). The order of the drink condition and running shoe type were randomized across the four trials. Participants ingested a total volume of 800 mL of a given drink during each experimental trial. The first bolus of the drink for the respective condition was 500 mL, and was consumed 20 min before commencing the exercise test (-20 min). The second bolus was 300 mL, and was ingested immediately after the second 8 min running stage during the 2 min rest interval. CHO consisted of a 10% carbohydrate solution with cyclic-dextrin (100% Cyclic-Dextrin; Myprotein; Northwich, UK) as the carbohydrate source and flavored with a peach sweetener (FlavDrops™; Myprotein). The quantity of carbohydrate ingested was provided to approximate a rate of 1.0 $g \cdot min^{-1}$ of exercise. KME consisted of 750 $mg \cdot kg^{-1}$ of body mass of a R-BD R-βHB KME (KetoneAid™ KE4; Falls Church, VA) mixed with water flavored with peach sweetener (FlavDrops™; Myprotein). The overall dose of the R-BD R-βHB KME was split into two boluses. The first drink ingested at -20 min provided 500 $mg \cdot kg^{-1}$, whereas 250 $mg \cdot kg^{-1}$ was provided in the second drink ingested upon completion of the second 8 min stage. This multiple bolus dosing strategy for R-BD R-βHB KME is commonly employed in similar studies [reviewed in (13)] to provide relatively stable and elevated circulating βHB concentrations throughout exercise. The volume of water ingested in KME was identical to that in CHO. CHO 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. All drinks were provided in opaque bottles but participants were informed that they would receive each drink on two occasions. To assess the

success of blinding to the drink conditions, on completion of the final experimental trial, participants were asked to identify the trials in which they believed they had received the R-BD R-βHB KME. Participants were also asked to identify the trial in which they believed they had ran for the longest duration during the ramp test to volitional exhaustion.

Participants were provided with commercially-available running shoes for the experimental trials. During PEG, participants wore the Nike Pegasus Turbo (Nike, Beaverton, OR) while during VAP, participants wore the Nike ZoomX Vaporfly Next% 3 (Nike). The Nike ZoomX Vaporfly Next% 3 was selected as the AFT shoe as it is the same brand featured in the seminal study on AFT, which demonstrated an ~4% improvement in RE over a more traditional, non-AFT running shoe (5). The Nike Pegasus Turbo was selected as the control shoe as it lacks a CFP, and its midsole is only partially composed of Pebax®, which is a primary feature of the Nike ZoomX Vaporfly Next% 3 midsole. The Nike ZoomX Vaporfly Next% 3 has a heel stack height of ~40 mm and a forefoot stack height of ~32 mm, resulting in an ~8 mm drop. In comparison, the Nike Pegasus Turbo has a heel stack height of ~32 mm and a forefoot stack height of ~22 mm, giving it an ~10 mm drop. The similarity in drop heights between the two models minimizes potential differences in stride patterns that might otherwise arise from variations in drop height. Participants were not blinded to the shoe type and were allowed to self-select their shoe size from US 6.5, 7.5, 8.5, 9.5, 10.5, or 11.5 during the first trial involving each shoe type, but were required to use the same size for the subsequent trial with that shoe. The average single shoe mass of PEG for sizes 6.5, 7.5, 8.5, 9.5, 10.5, and 11.5 was 219 g, 237 g, 239 g, 262 g, 272 g, and 283 g, respectively. For VAP, the corresponding average single shoe mass to sizes 6.5, 7.5, 8.5, 9.5, 10.5, and 11.5 was 153 g, 171 g, 183 g, 196 g, 207 g, and 222 g, respectively. The average difference in mass between the shoe types was 63 g. To maintain ecological validity, no adjustments were made to the shoe mass. The total distance ran in each pair of shoes across the entire study ranged from 66 to 133 km.

Gastrointestinal tolerability

Type and severity of gastrointestinal (GI) symptoms were measured using a 12-item questionnaire (24) for each condition before the start of exercise and on completion of the ramp test to

volitional exhaustion. Symptom severity was rated on a Likert scale between 0 (none) and 8 (unbearable) which were subsequently categorized as none (0), mild (1 or 2), moderate (3 or 4), or severe (5+). Total symptom load at pre-exercise and peak exercise was calculated as the sum of participant scores for all symptoms as described previously (24).

Statistical analyses

Statistical analysis was performed in GraphPad Prism v.10.3 (GraphPad Software Inc., La Jolla, CA). Data are presented as mean ± SD unless otherwise stated. Differences in body mass, hydration status, weekly training sessions, weekly distance, and average HR (AHR) and maximum HR (MHR) during training were compared between conditions using a one-way repeated measures ANOVA. A two-way (time-condition) repeated measures ANOVA was performed to determine differences in blood βHB, blood glucose, and blood lactate concentration (time levels: -20 min, 0 min, Stage 1, Stage 2, Stage 3, Stage 4, Stage 5, and peak exercise), RE, $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER, relative $\dot{V}O_2$, HR, and RPE (time levels: Stage 1, Stage 2, Stage 3, Stage 4, Stage 5). When a main effect of condition or a time-condition interaction effect was indicated, *post-hoc* testing was performed with Tukey's correction. Multiplicity-adjusted *P* values are reported to compare PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME at the respective time points. A one-way repeated-measures ANOVA was used to compare $\dot{V}O_{2peak}$, HR_{peak}, and TTE between conditions. Sphericity was not assumed, and the Greenhouse–Geisser correction was applied to all ANOVA analyses. Standardized estimates of ES were calculated using the ratio of the mean difference to the pooled SD for all primary and secondary outcome variables and are presented as the adjusted Hedges' *g*. Differences between conditions in GI symptoms were examined using Friedman's test with Dunn's *post-hoc* correction. The significance level was set at $\alpha \leq 0.05$ for all tests.

RESULTS

Body mass and hydration status

Body mass was not significantly different (P=0.575, ES=0.00-0.03) between PEG+CHO (66.5±10.0 kg), PEG+KME (66.2±9.7 kg), VAP+CHO (66.4±10.1 kg), and VAP+KME (66.4±10.1 kg). There was also no significant difference in hydration status (P=0.860, ES=0.00-0.20) between PEG+CHO (1.010±0.005 $g \cdot mL^{-1}$), PEG+KME (1.010±0.005 $g \cdot mL^{-1}$), VAP+CHO (1.009±0.006 $g \cdot mL^{-1}$), and VAP+KME (1.009±0.005 $g \cdot mL^{-1}$). Body mass and hydration status for male and female participants during each condition are presented in Supplementary Table 1. The average number of training sessions performed each week during the study period was 4.3±1.1, with a corresponding average weekly distance of 47.3±16.1 km. The AHR and MHR recorded during these sessions were 149±8 bpm and 167±8 bpm, respectively. There was no significant difference between conditions in the number of training sessions (P=0.082, ES=0.08–0.74), weekly distance (P=0.125, ES=0.07–0.74), AHR (P=0.448, ES=0.00–0.36), or MHR (P=0.582, ES=0.00–0.22). There was no trial order effect found for number of training sessions (P=0.870), weekly distance (P=0.790), AHR (P=0.382), or MHR (P=0.704).

Blood βHB, glucose, and lactate concentration

A time–condition interaction effect (P<0.001) was found for blood βHB concentration (Figure 2A). With the exception of -20 min, blood βHB concentration was significantly higher at all time points in both PEG+KME and VAP+KME compared to PEG+CHO and VAP+CHO (all P<0.001, ES=3.71-5.53). There were no significant differences in blood βHB concentration between PEG+KME and VAP+KME at any time point (all P>0.05, ES=0.00-0.13). A time-condition interaction effect was found for blood glucose concentration (P<0.001) (Figure 2B) and blood lactate concentration (P=0.020) (Figure 2C). At peak exercise, blood lactate concentration was significantly lower in PEG+KME (5.8±2.7 mM) compared to PEG+CHO (7.3±3.0 mM; P=0.040, ES=0.51) and VAP+CHO (7.1±3.0 mM; P=0.022, ES=0.45). Blood βHB, glucose, and lactate concentrations for male and female participants at each submaximal running stage and at peak exercise during each condition are presented in Supplementary Table 2.

Submaximal exercise

A significant effect of time ($P<0.001$) and condition ($P<0.001$) was found for RE, but there was no time-condition interaction effect ($P=0.336$) (Figure 3). RE was lower during the third submaximal running stage in VAP+CHO compared to PEG+CHO (mean difference of 7.4, 95% CI=1.8-13.0 $mL \cdot kg^{-1} \cdot km^{-1}$, $P=0.008$, ES=0.81), in VAP+KME compared to PEG+CHO (mean difference of 6.0, 95% CI=1.5-10.6 $mL \cdot kg^{-1} \cdot km^{-1}$, $P=0.008$, ES=0.61), in VAP+CHO compared to PEG+KME (mean difference of 7.4, 95% CI=1.2-13.6 $mL \cdot kg^{-1} \cdot km^{-1}$, $P=0.017$, ES=0.75), and in VAP+KME compared to PEG+KME (mean difference of 6.1, 95% CI=3.1-9.1 $mL \cdot kg^{-1} \cdot km^{-1}$, $P<0.001$, ES=0.57). During the fourth submaximal running stage, RE was lower in VAP+CHO compared to PEG+CHO (mean difference of 6.7, 95% CI=1.2-12.2 $mL \cdot kg^{-1} \cdot km^{-1}$, $P=0.015$, ES=0.72), in VAP+KME compared to PEG+CHO (mean difference of 5.1, 95% CI=1.1-9.1 $mL \cdot kg^{-1} \cdot km^{-1}$, $P=0.010$, ES=0.53), in VAP+CHO compared to PEG+KME (mean difference of 8.1, 95% CI=4.3-11.9 $mL \cdot kg^{-1} \cdot km^{-1}$, $P<0.001$, ES=0.84), and in VAP+KME compared to PEG+KME (mean difference of 6.5, 95% CI=2.7-10.4 $mL \cdot kg^{-1} \cdot km^{-1}$, $P<0.001$, ES=0.66). During the final submaximal running stage, RE was lower in VAP+KME compared to PEG+CHO (mean difference of 5.8, 95% CI=0.9-10.7 $mL \cdot kg^{-1} \cdot km^{-1}$, $P=0.017$, ES=0.56), in VAP+CHO compared to PEG+KME (mean difference of 6.0, 95% CI=2.8-9.1 $mL \cdot kg^{-1} \cdot km^{-1}$, $P<0.001$, ES=0.60), and in VAP+KME compared to PEG+KME (mean difference of 6.5, 95% CI=2.4-10.6 $mL \cdot kg^{-1} \cdot km^{-1}$, $P=0.002$, ES=0.66). No significant differences were found between PEG+CHO and PEG+KME (all $P>0.05$, ES=0.00-0.14) or VAP+CHO and VAP+KME (all $P>0.05$, ES=0.05-0.17) across the five submaximal running stages.

A significant time-condition interaction effect was found for $\dot{V}E$ ($P=0.032$) (Figure 4A) and $\dot{V}CO_2$ ($P=0.011$) (Figure 4C), with progressive increases between running speeds in all conditions. A significant main effect of time ($P<0.001$) and condition ($P<0.001$) was found for $\dot{V}O_2$ with progressive increases between running speeds in all conditions also, but there was no time-condition interaction effect (Figure 4B). A time-condition interaction effect was found for RER ($P<0.001$) (Figure 4D). Main effects of time ($P<0.001$) and condition ($P<0.001$) were found for relative $\dot{V}O_2$, but there was no time–

condition interaction effect (P=0.087). A significant time-condition interaction effect (P<0.001) was found when relative $\dot{V}O_2$ was expressed as a percentage of $\dot{V}O_{2peak}$ (Table 1). A main effect of time (P<0.001) was found for HR, but when expressed as a percentage of HR_{peak}, there was a significant time-condition interaction effect (P<0.001). There was a main effect of time (P<0.001) for RPE, but no main effect of condition (P=0.051) or time-condition interaction effect (P=0.598) (Table 1). $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER, RE, and RPE for male and female participants at each submaximal running stage during each condition are presented in Supplementary Tables 3 and 4. $\dot{V}O_2$, $\%\dot{V}O_{2peak}$, HR, and $\%HR_{peak}$ for male and female participants at each submaximal running stage during each condition are presented in Supplementary Tables 5 and 6.

Ramp test to exhaustion

$\dot{V}O_{2peak}$ was not significantly different between PEG+CHO (59.5±7.2 $mL \cdot kg^{-1} \cdot min^{-1}$) and PEG+KME (59.2±7.3 $mL \cdot kg^{-1} \cdot min^{-1}$, P=0.958, ES=0.04), PEG+CHO and VAP+CHO (60.7±7.1 $mL \cdot kg^{-1} \cdot min^{-1}$; P=0.056, ES=0.16), PEG+CHO and VAP+KME (60.3±7.4 $mL \cdot kg^{-1} \cdot min^{-1}$; P=0.413, ES=0.11), PEG+KME and VAP+CHO (P=0.157, ES=0.20), PEG+KME and VAP+KME (P=0.209, ES=0.15), or VAP+CHO and VAP+KME (P=0.824, ES=0.05) (Figure 5A). There were no significant differences in HR_{peak} (P=0.517, ES=0.00-0.11) between PEG+CHO (188±9 bpm), PEG+KME (187±8 bpm), VAP+CHO (187±10 bpm), and VAP+KME (187±8 bpm) (Figure 5B). TTE was significantly lower during PEG+CHO (357±140 s) compared to VAP+CHO (381±125 s; P=0.023, ES=0.18). TTE was also significantly lower during PEG+KME compared to PEG+CHO (357±140 s; P=0.008, ES=0.19), VAP+CHO (P<0.001, ES=0.40) and VAP+KME (375±125 s; P<0.001, ES=0.35) (Figure 5C). $\dot{V}O_{2peak}$, HR_{peak}, and TTE for male and female participants during each condition are presented in Supplementary Table 1.

GI tolerability and hormonal contraception

The prevalence of GI symptoms, recorded 5 min prior to the start of exercise and 5 min after the cessation of exercise in PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME are presented in Table 2. Total symptom load prior to the start of exercise was 9 in PEG+CHO, 17 in PEG+KME, 6 in

VAP+CHO, and 18 in VAP+KME. Total symptom load at peak exercise was 26, 85, 42, and 50 in PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME, respectively. Symptom load was significantly different between conditions at peak exercise (P=0.047), but after adjustment for multiple comparisons, *post hoc* analyses did not show any differences between PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME.

Blinding

Thirteen participants (72%) correctly identified the condition in which they ran for the longest duration in the ramp test to volitional exhaustion. Fourteen participants (78%) correctly identified the two trials in which they received the R-BD R-βHB KME based on taste alone, with three participants (17%) correctly identifying one trial. One participant (6%) failed to identify at least one trial in which they received the R-BD R-βHB KME based on taste alone. Based on any factor other than taste, ten participants (56%) correctly identified the two trials in which they received the R-BD R-βHB KME, two participants (11%) correctly identified one trial, while six participants (33%) could not differentiate between trials or chose incorrectly.

DISCUSSION

This study examined the effect of AFT and acute ingestion of a R-BD R-βHB KME both individually and in combination, on the RE of trained male and female middle- and long-distance runners. A significant improvement in RE of 2.5% to 4.0% was found during the latter three submaximal running stages in VAP+CHO and VAP+KME compared to PEG+CHO and PEG+KME but no significant differences were found between PEG+CHO and PEG+KME, or VAP+CHO and VAP+KME, across all five submaximal running stages. TTE was significantly improved by 14.0% and 15.8% during VAP+CHO and VAP+KME compared to PEG+KME, respectively and by 7.0% during VAP+CHO compared to PEG+CHO. While AFT significantly improved RE, there was no synergistic effect between AFT and acute ingestion of the R-BD R-βHB KME, likely due to the absence of differences between CHO and KME across both shoe types.

Both AFT (5) and acute ingestion of the R-BD R-βHB KME (18) have been found in independent studies to improve RE by ~4%, likely through different mechanisms. Running shoes featuring AFT may enhance RE by improving biomechanical properties and energy return (6–8), whereas the R-BD R-βHB KME may improve RE by providing an additional substrate for oxidation in skeletal muscle and other tissues that is more efficient in the production of ATP than glucose which in turn, may also reduce oxygen cost and/or the reliance on glucose metabolism during exercise (13). However, their combined effects have not yet been examined. In the present study, the lack of a combined effect is largely due to the trivial effect of the R-BD R-βHB KME, as demonstrated by the average change in RE between PEG+CHO and PEG+KME, which ranged from a 0.5% improvement to a 0.7% decrement. Similarly, for VAP+CHO and VAP+KME, the differences in RE ranged from a 0.8% decrement to a 0.3% improvement across the submaximal running stages.

The absence of significant differences between CHO and KME conditions means that acute ingestion of the R-BD R-βHB KME in the present study had no effect on RE, a finding that is in direct contrast to our previous study that found an average improvement of 4.1% in RE in male middle- and long-distance runners at 10 to 14 $km \cdot h^{-1}$ after ingestion of 750 $mg \cdot kg^{-1}$ body mass of the R-BD R-βHB KME when compared to ingestion of CHO (18). The reasons for the lack of replication of the effect of the R-BD R-βHB KME on RE are unclear, given that the study protocol was practically identical, with

the exception of the participants, the commercial supplier of the R-BD R-βHB KME, and minor adjustments to the time of day at which the exercise was performed. The blood concentration of βHB immediately prior to exercise was notably higher in the present study, reaching 3.6 mM and 3.5 mM during PEG+KME and VAP+KME, respectively, compared to 2.6 mM in the ketone only condition in our previous work (18). Similarly, βHB concentrations during exercise were higher, ranging from 2.4 to 2.8 mM and 2.4 to 2.9 mM during PEG+KME and VAP+KME, respectively, compared to 1.7 to 2.4 mM during exercise in our previous study. The higher βHB concentrations are partly due to the inclusion of female participants, who consistently had a higher concentration of βHB than male participants across the range of submaximal running stages (Supplementary Table 2). Nonetheless, it remains unclear how the concentration of βHB influences the ergogenic potential or metabolic effects of EKS during exercise (13), and is unlikely that average differences of 0.5 to 0.9 mM across the five submaximal running stages account for the complete absence of an effect on RE compared to our previous study. Our sample size was not powered to investigate whether these differences were indeed sex-related, but we performed exploratory analyses on the male and female participants as separate datasets using the same two-way (time-condition) repeated measures ANOVA that was used for the primary analysis. Again, there were no significant differences in RE between CHO and KME conditions for either male (ANOVA condition effect: all $P>0.05$, ES=0.08-0.38) or female (ANOVA condition effect: all $P>0.05$, ES=0.02-0.31) participants.

In the field of sport and exercise science, there is increasing attention on the challenge of replicating the findings of previous studies (25). An extensive study, currently in pre-print, attempted to replicate twenty-five studies in this field and found that only 56% or 14/25 achieved statistical significance as a replication of the original finding. Moreover, only 28% or 7/25 achieved both statistical significance and an ES similar to the original study (26). In this context, despite the abovementioned approaches to experimental standardization and control, it is perhaps unsurprising that we did not replicate our previous findings of improved RE with acute ingestion of the R-BD R-βHB KME compared to CHO. Separate to the challenge of replication, it is pertinent to consider the mechanistic basis for whether acute ingestion of EKS would be expected to alter RE. The hypothesis is largely based on the observations that KB can be used as a substrate by skeletal muscle (14, 15), and

365 can theoretically provide a more efficient source of ATP compared to glucose, which may in turn reduce
366 the oxygen cost of exercise in the presence of "acute nutritional ketosis" (13). However, the
367 contribution of βHB oxidation to energy provision during exercise is typically reported to be ~5% (16,
368 17), which may be too minor a contribution to meaningfully and consistently impact oxygen utilization.
369 Indeed, *in vitro* studies on permeabilized muscle fibers and isolated mitochondria from skeletal muscle
370 demonstrate that KB make a minimal contribution to mitochondrial respiration, particularly when other
371 substrates (e.g., pyruvate) are readily-available, and, therefore, are unlikely to be preferentially used
372 over glucose in skeletal muscle (27, 28). For example, the addition of pyruvate to ketone body-
373 supported respiration in isolated skeletal muscle mitochondria increased respiration, whereas KB had a
374 negligible effect on pyruvate-supported respiration (28). Follow-up studies will be required to fully
375 explore the potential impact of ingestion of EKS on oxygen cost, RE, and related parameters in
376 prolonged exercise challenges under different dosing and co-ingestion strategies.
377 Despite the lack of a synergistic effect between AFT and acute ingestion of the R-BD R-βHB
378 KME, RE was significantly improved in both trials involving AFT (i.e., VAP+CHO and VAP+KME)
379 compared to the control running shoes (i.e., PEG+CHO and PEG+KME) during the latter submaximal
380 running stages. The lack of difference in RE during the first two submaximal running stages may be
381 partially explained by reduced elastic properties of running shoes featuring AFT at lower speeds (29,
382 30). Improvements in RE of only 0.9% and 1.4% were found for recreational runners at 10 $km \cdot h^{-1}$ and
383 12 $km \cdot h^{-1}$, respectively, with the former being non-significant (29). The lack of difference may also be
384 due to other factors such as the absence of a warm-up and changes in gait due to running speeds being
385 lower than typical training intensities (31).
386 The improvements in RE from AFT in the present study ranged from 2.5% to 4.0%.
387 Improvements in RE ranging from 2.7% to 4.2% have been reported in previous studies comparing AFT
388 and non-AFT running shoes (5, 11, 12, 32). However, caution is warranted when drawing parallels
389 between studies, or making broad comments about the magnitude of effect across studies, due to
390 differences in the running speeds used in previous investigations, which were typically higher (14 to 18
391 $km \cdot h^{-1}$) than those in the present study, as well as notable differences in the characteristics of both the

AFT and non-AFT running shoes used in this study compared to those in previous investigations. In one study involving highly-trained male and female runners, when shoe mass of VAP was adjusted to match the control shoe, the improvement in RE was reduced from 4.4% to 2.9% (11). This improvement is akin to the improvement found in the present study, despite not adjusting for shoe mass. Our decision not to control shoe mass was made to improve the translatability and generalizability of the results, with both shoe models being commercially-available at the time of the study. Although the average difference in shoe mass between the AFT and non-AFT running shoes in the present study was only ~63 g, it is important to consider these differences when interpreting the magnitude of change in RE, as $\dot{V}O_2$ can be elevated by ~1% per 100 g increase in shoe mass (33). Features of AFT beyond shoe mass, such as stiffness, midsole resilience, and energy return, likely contributed to the improvements in RE observed in the present study. Quantifying these characteristics requires specialized methodologies, which were not available for this study and are often not reported in studies comparing AFT and non-AFT running shoes. This absence of detail is acknowledged as a limitation of the present work, but is also a broader limitation in the interpretation and comparison of findings across this field.

The inclusion of both male and female middle- and long-distance runners is a strength of this study, although the data were not sufficiently powered to allow for a between-sex comparison of the response to each condition, which constitutes a limitation. While between sex-differences in substrate utilization during prolonged exercise are well-established (34, 35), we were unable to measure substrate utilization in response to acute ingestion of the R-BD R-βHB KME. This inability is due to these calculations not being valid under conditions of ketosis, as well as the RER of βHB (~1.00) and acetoacetate (~0.89) being similar to glucose oxidation (1.00) (13, 36). The need for invasive techniques such as muscle biopsies and tracers would also require considerable modifications to the exercise protocol. By contrast, male and female runners experience a similar response to AFT across a range of speeds (11), with stride patterns and differences in gait likely contributing to changes in RE to a greater extent than biological sex.

Variations in substrate utilization have been attributed, at least in part, to menstrual cycle phase and hormonal contraceptive use due to fluctuations in circulating estrogen and progesterone concentrations (34). In the present study, sex hormone concentrations were unknown and not

standardized as this would have required the exclusion of either hormonal contraceptive users or normally menstruating females, a consideration for which there is limited supporting evidence in exercise performance (22). Additionally, menstrual cycle length can vary between females by 14 d, and cycle phase length (i.e., follicular phase and luteal phase) can vary by ~5 d within females (37). Standardizing the menstrual cycle phase would, therefore, have prevented the completion of exercise trials across consecutive weeks, and instead required completion in the same phase of consecutive cycles i.e., a time span of ~12 weeks. Such a design would have elicited attendant concerns about the impact of habitual training on RE over such a time frame. The employed experimental design, therefore, prioritized minimizing the impact of habitual exercise training, which is more likely to have a meaningful effect on RE than menstrual cycle phase and hormonal contraceptive use (2, 22, 23).

In summary, AFT, but not acute ingestion of the R-BD R-βHB KME improved RE in a cohort of trained male and female middle- and long-distance runners at submaximal running speeds. The lack of improvement in RE in response to acute ingestion of the R-BD R-βHB KME limits the ability to conclude whether combining a dietary supplement with AFT could provide a greater benefit on RE than either condition alone. Future research should investigate how other dietary supplements or bioactive compounds may interact with the mechanical and/or biomechanical advantages in RE provided by AFT, and whether such combinations could produce a synergistic effect.

ACKNOWLEDGEMENTS

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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FIGURE LEGENDS

Figure 1. Overview of each study visit and the exercise protocol. The speeds for male (M) and female (F) participants during the exercise test are indicated on the left Y-axes, with corresponding labels above each axis.

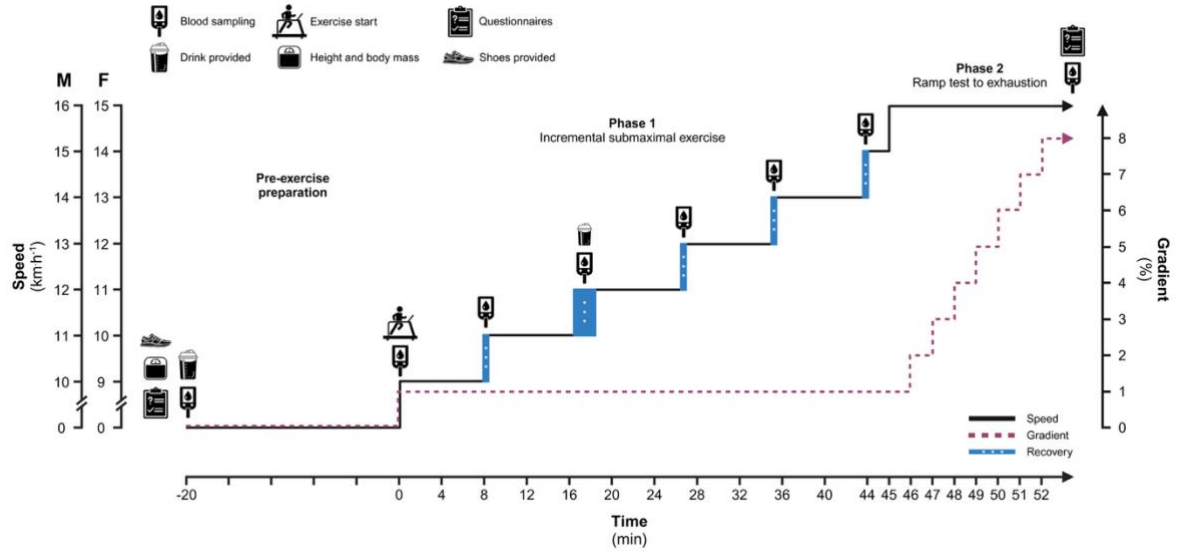


Figure 2. Blood βHB (A), glucose (B), and lactate (C) concentration during PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME. Data are presented as mean values, with error bars representing the SD. *, P<0.05 PEG+CHO versus PEG+KME; **, P<0.01 PEG+CHO versus PEG+KME; ***, P<0.001 PEG+CHO versus PEG+KME; ‡‡, P<0.01 PEG+CHO versus VAP+KME; ‡‡‡, P<0.001 PEG+CHO versus VAP+KME; ¥, P<0.05 PEG+KME versus VAP+CHO; ¥¥ P<0.01 PEG+KME versus VAP+CHO; ¥¥¥ P<0.001 PEG+KME versus VAP+CHO; ¤¤ P<0.01 VAP+CHO versus VAP+KME; ¤¤¤ P<0.001 VAP+CHO versus VAP+KME.

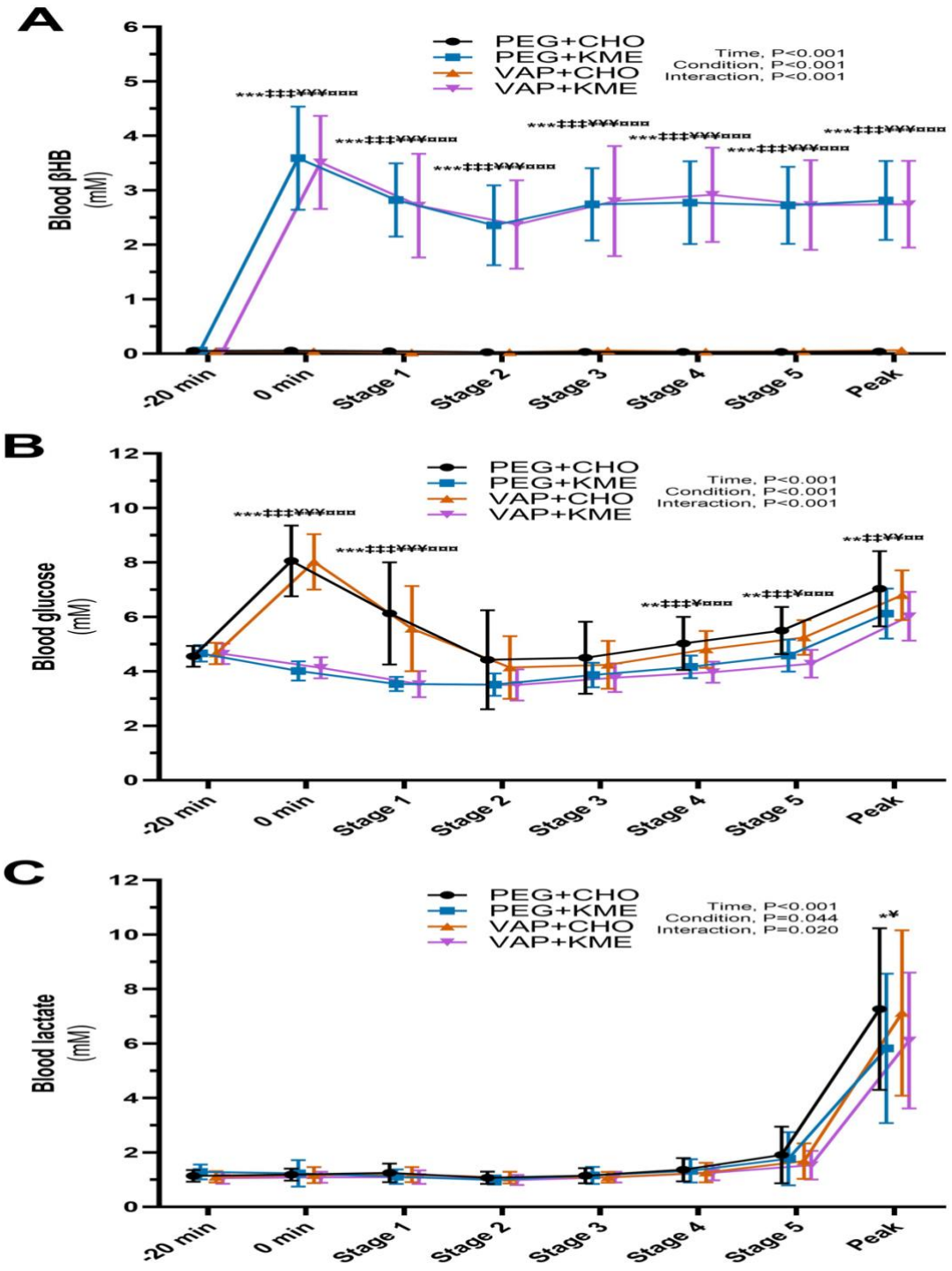


Figure 3. RE at each submaximal running stage during PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME. Data are presented as mean values, with error bars representing the SD. † P<0.05 PEG+CHO versus VAP+CHO; †† P<0.01 PEG+CHO versus VAP+CHO; ‡, P<0.05 PEG+CHO versus VAP+KME; ‡‡, P<0.01 PEG+CHO versus VAP+KME; ¥, P<0.05 PEG+KME versus VAP+CHO; ¥¥¥ P<0.001 PEG+KME versus VAP+CHO; § P<0.05 PEG+KME versus VAP+KME; §§ P<0.01 PEG+KME versus VAP+KME; §§§ P<0.001 PEG+KME versus VAP+KME.

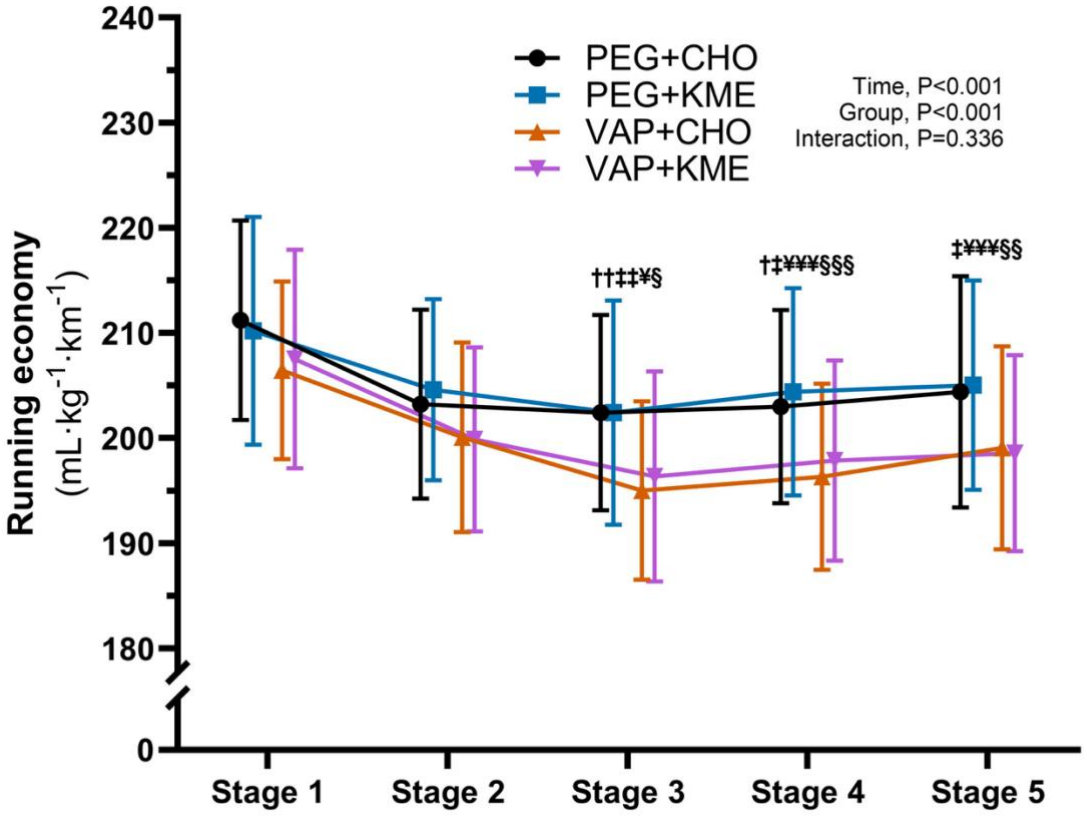


Figure 4. Minute $\dot{V}E$ (A), $\dot{V}O_2$ (B), $\dot{V}CO_2$ (C) and RER (D) at each submaximal running stage during PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME. Data are presented as mean values, with error bars representing the SD. *, P<0.05 PEG+CHO versus PEG+KME; **, P<0.01 PEG+CHO versus PEG+KME; † P<0.05 PEG+CHO versus VAP+CHO; †† P<0.01 PEG+CHO versus VAP+CHO; ‡, P<0.05 PEG+CHO versus VAP+KME; ‡‡, P<0.01 PEG+CHO versus VAP+KME; ‡‡‡, P<0.001 PEG+CHO versus VAP+KME; $^{¥}$, P<0.05 PEG+KME versus VAP+CHO; $^{¥¥}$ P<0.01 PEG+KME versus VAP+CHO; $^{¥¥¥}$ P<0.001 PEG+KME versus VAP+CHO; § P<0.05 PEG+KME versus VAP+KME; §§ P<0.01 PEG+KME versus VAP+KME; $^{¤}$ P<0.05 VAP+CHO versus VAP+KME; $^{¤¤¤}$ P<0.001 VAP+CHO versus VAP+KME.

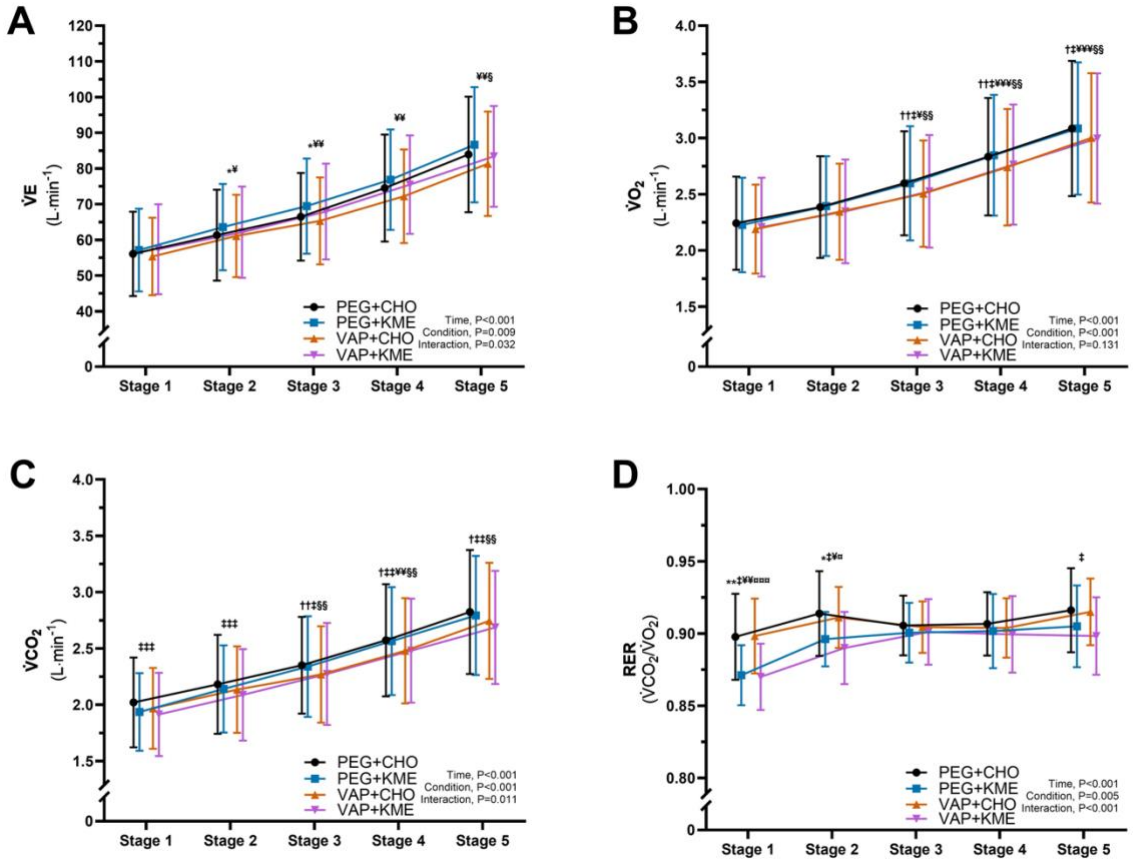
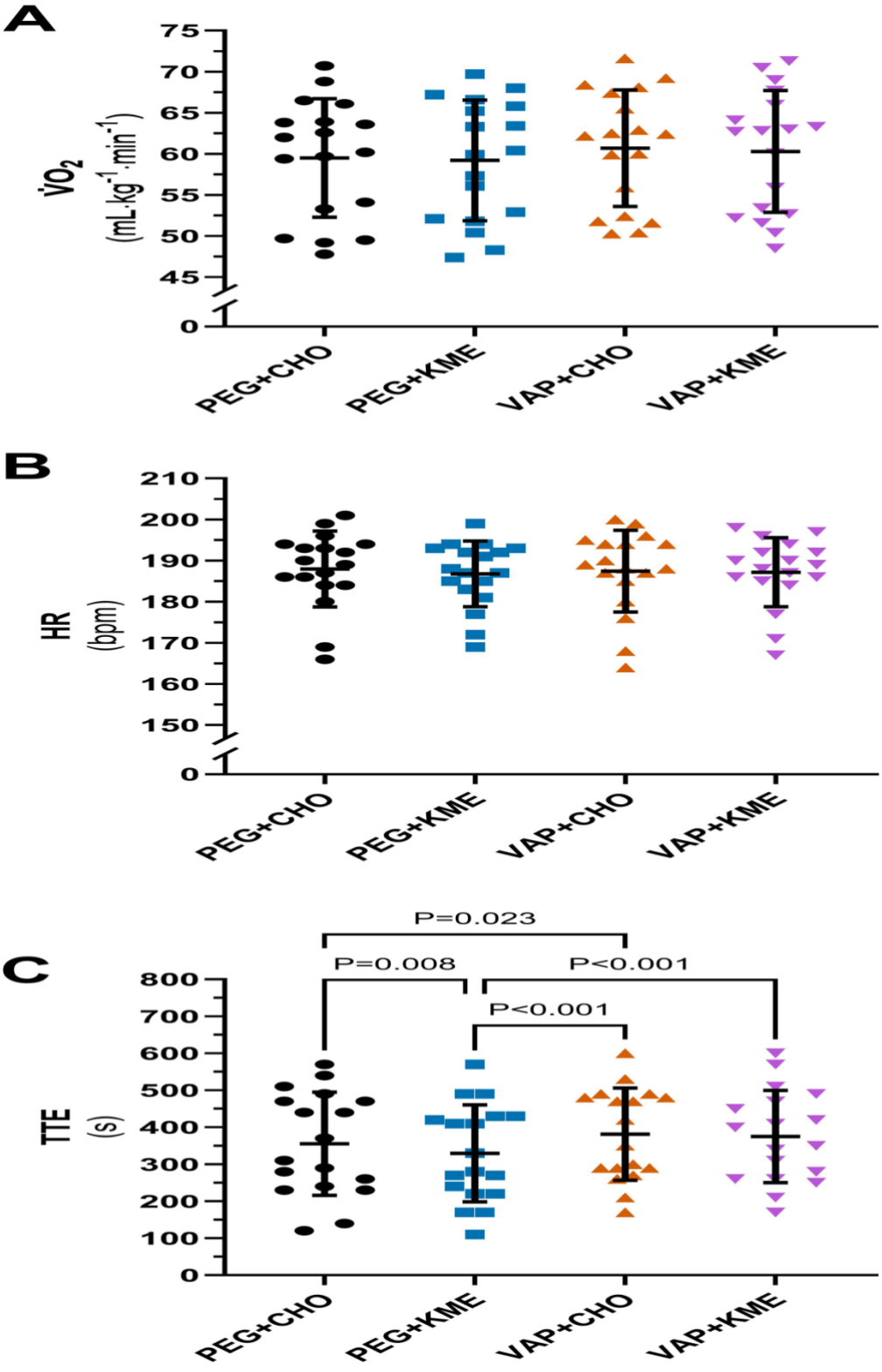


Figure 5. $\dot{V}O_{2peak}$ (A), HR_{peak} (B), and TTE (C) during PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME. Filled circles, squares, triangles, and inverted triangles represent the individual data points from PEG+CHO, PEG+KME, VAP+CHO, and VAP+KME, respectively, with error bars representing SD



SUPPLEMENTARY DIGITAL CONTENT

Supplementary Table 1. Descriptive statistics and peak exercise responses for male and female participants during each condition.

Condition	Group	Body mass (kg)	Hydration ($g \cdot mL^{-1}$)	$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	HR_{peak} (bpm)	TTE (s)
PEG+CHO	Male	73.5±7.7	1.010±0.004	64.4±3.6	184±10	429±113
	Female	57.7±2.9	1.009±0.007	53.3±5.6	192±6	264±116
PEG+KME	Male	73.1±7.3	1.012±0.005	64.6±3.8	184±9	402±109
	Female	57.7±2.9	1.007±0.004	52.4±4.2	190±6	239±97
VAP+CHO	Male	73.5±7.7	1.011±0.006	65.8±3.8	184±11	448±101
	Female	57.5±2.9	1.006±0.005	54.4±4.6	192±5	298±101
VAP+KME	Male	73.6±7.5	1.009±0.004	65.8±3.7	184±10	447±98
	Female	57.5±2.9	1.008±0.006	53.4±4.4	191±5	285±94

Data are presented as mean±SD. Male, n=10; Female, n=8.

Supplementary Table 2. Blood βHB, glucose, and lactate concentration for male and female participants at each submaximal stage and at peak exercise during each condition.

	Condition	Group	-20 min	0 min	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Peak
βHB (mM)	PEG+CHO	Male	0.1±0.1	0.1±0.1	0.1±0.1	0.0±0.1	0.0±0.1	0.0±0.1	0.0±0.0	0.0±0.1
		Female	0.0±0.1	0.1±0.1	0.0±0.1	0.0±0.1	0.0±0.1	0.0±0.1	0.1±0.1	0.1±0.1
	PEG+KME	Male	0.1±0.1	3.7±0.7	2.7±0.7	2.3±0.7	2.6±0.7	2.6±0.8	2.6±0.7	2.7±0.8
		Female	0.0±0.0	3.4±1.2	3.0±0.7	2.5±0.8	2.9±0.6	3.0±0.6	2.9±0.7	2.9±0.7
	VAP+CHO	Male	0.1±0.1	0.0±0.1	0.0±0.0	0.0±0.0	0.1±0.1	0.0±0.1	0.1±0.1	0.1±0.1
		Female	0.0±0.1	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.1	0.0±0.1	0.0±0.1	0.0±0.1
	VAP+KME	Male	0.1±0.1	3.5±0.9	2.3±1.0	2.1±0.8	2.5±1.1	2.6±0.9	2.4±0.8	2.4±0.8
		Female	0.0±0.0	3.5±0.8	3.2±0.6	2.7±0.7	3.2±0.8	3.3±0.7	3.2±0.6	3.2±0.6
Glucose (mM)	PEG+CHO	Male	4.6±0.3	8.0±1.1	5.7±1.7	4.0±1.7	4.1±1.0	4.7±0.8	5.2±0.6	6.6±1.1
		Female	4.4±0.5	8.2±1.6	6.6±2.1	4.9±2.0	5.0±1.5	5.4±1.1	5.9±1.0	7.5±1.6
	PEG+KME	Male	4.6±0.3	4.0±0.2	3.4±0.3	3.3±0.3	3.6±0.4	4.0±0.4	4.3±0.6	6.1±1.0
		Female	4.7±0.3	4.0±0.5	3.7±0.1	3.8±0.4	4.2±0.4	4.4±0.4	4.9±0.4	6.2±0.9
	VAP+CHO	Male	4.6±0.2	7.9±1.0	5.4±1.6	3.9±1.1	4.1±1.0	4.7±0.8	5.1±0.6	6.5±0.9
		Female	4.7±0.6	8.2±1.1	5.8±1.6	4.4±1.2	4.4±0.8	5.0±0.5	5.4±0.6	7.2±0.9
	VAP+KME	Male	4.6±0.4	4.2±0.3	3.4±0.4	3.2±0.6	3.5±0.5	3.8±0.4	4.1±0.4	5.8±0.9
		Female	4.7±0.4	4.0±0.5	3.7±0.5	3.9±0.3	4.1±0.4	4.2±0.3	4.6±0.5	6.3±0.9
Lactate (mM)	PEG+CHO	Male	1.2±0.2	1.3±0.2	1.3±0.4	1.0±0.2	1.2±0.3	1.4±0.4	1.8±0.9	7.5±3.3
		Female	1.1±0.2	1.1±0.2	1.2±0.2	1.1±0.2	1.1±0.3	1.3±0.5	2.1±1.2	7.0±2.8
	PEG+KME	Male	1.3±0.3	1.2±0.4	1.1±0.3	0.9±0.2	1.2±0.4	1.3±0.5	1.7±1.3	6.3±3.1
		Female	1.3±0.3	1.3±0.6	1.2±0.3	1.1±0.2	1.1±0.2	1.3±0.3	1.8±0.5	5.3±2.2
	VAP+CHO	Male	1.1±0.2	1.2±0.2	1.1±0.3	1.0±0.2	1.1±0.2	1.3±0.4	1.6±0.7	7.7±3.3
		Female	1.1±0.2	1.2±0.4	1.3±0.3	1.1±0.2	1.1±0.2	1.3±0.2	1.9±0.7	6.4±2.7
	VAP+KME	Male	1.1±0.2	1.1±0.1	1.0±0.2	1.0±0.2	1.1±0.2	1.2±0.3	1.4±0.6	6.0±2.7
		Female	1.0±0.2	1.1±0.3	1.3±0.2	1.0±0.1	1.1±0.2	1.4±0.3	1.7±0.4	6.2±2.4

Data are presented as mean±SD. Male, n=10; Female, n=8.

Supplementary Table 3. $\dot{V}E$, $\dot{V}O_2$, and $\dot{V}CO_2$ for male and female participants at each submaximal stage during each condition.

	Condition	Group	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
$\dot{V}E$ ($L \cdot min^{-1}$)	PEG+CHO	Male	61.6±10.4	67.1±11.8	72.0±10.9	81.7±12.9	91.1±14.4
		Female	49.1±10.0	54.1±10.4	59.5±10.7	65.5±12.9	75.1±14.4
	PEG+KME	Male	61.4±9.8	69.1±10.0	75.0±13.1	83.4±13.0	93.8±15.0
		Female	51.8±12.1	56.7±11.4	62.5±10.8	68.8±11.3	77.7±13.4
	VAP+CHO	Male	61.1±9.4	67.5±9.5	72.4±9.6	79.5±10.8	89.1±12.9
		Female	48.1±8.1	53.1±8.6	56.5±9.0	63.1±9.8	71.6±10.5
	VAP+KME	Male	61.5±12.3	67.5±11.8	73.2±12.4	80.3±12.7	88.3±13.1
		Female	52.1±11.7	55.5±11.3	61.3±12.4	69.5±13.5	77.2±13.6
$\dot{V}O_2$ ($L \cdot min^{-1}$)	PEG+CHO	Male	2.560±0.248	2.724±0.300	2.952±0.272	3.226±0.336	3.536±0.379
		Female	1.846±0.120	1.965±0.137	2.154±0.148	2.343±0.162	2.521±0.202
	PEG+KME	Male	2.531±0.291	2.733±0.274	2.979±0.338	3.269±0.298	3.546±0.324
		Female	1.845±0.144	1.971±0.116	2.122±0.127	2.321±0.127	2.511±0.163
	VAP+CHO	Male	2.502±0.213	2.677±0.247	2.872±0.273	3.143±0.299	3.444±0.341
		Female	1.799±0.094	1.930±0.111	2.049±0.133	2.239±0.138	2.451±0.171
	VAP+KME	Male	2.539±0.293	2.695±0.310	2.902±0.328	3.169±0.335	3.435±0.360
		Female	1.793±0.083	1.913±0.084	2.056±0.129	2.258±0.155	2.447±0.173
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	PEG+CHO	Male	2.327±0.234	2.510±0.284	2.678±0.261	2.945±0.316	3.230±0.347
		Female	1.639±0.125	1.771±0.137	1.942±0.132	2.109±0.179	2.316±0.224
	PEG+KME	Male	2.188±0.226	2.442±0.226	2.669±0.317	2.933±0.288	3.190±0.331
		Female	1.621±0.139	1.765±0.089	1.928±0.098	2.105±0.125	2.298±0.185
	VAP+CHO	Male	2.243±0.215	2.429±0.232	2.596±0.252	2.839±0.279	3.125±0.334
		Female	1.624±0.098	1.767±0.102	1.860±0.138	2.031±0.139	2.269±0.197
	VAP+KME	Male	2.188±0.257	2.396±0.258	2.618±0.288	2.828±0.297	3.064±0.320
		Female	1.572±0.081	1.703±0.091	1.844±0.102	2.046±0.130	2.214±0.152

Data are presented as mean±SD. Male, n=10; Female, n=8.

Supplementary Table 4. RER, RE, and RPE for male and female participants at each submaximal stage during each condition.

	Condition	Group	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
RER ($\dot{V}CO_2/\dot{V}O_2$)	PEG+CHO	Male	0.91±0.03	0.92±0.02	0.91±0.02	0.91±0.02	0.91±0.02
		Female	0.89±0.03	0.90±0.03	0.90±0.02	0.90±0.03	0.92±0.04
	PEG+KME	Male	0.87±0.02	0.90±0.02	0.89±0.02	0.90±0.03	0.90±0.02
		Female	0.88±0.02	0.90±0.02	0.91±0.02	0.91±0.02	0.91±0.03
	VAP+CHO	Male	0.90±0.03	0.91±0.02	0.90±0.02	0.90±0.02	0.91±0.02
		Female	0.90±0.02	0.92±0.02	0.91±0.02	0.91±0.02	0.93±0.02
	VAP+KME	Male	0.86±0.03	0.89±0.03	0.91±0.03	0.89±0.03	0.89±0.03
		Female	0.88±0.02	0.89±0.02	0.90±0.02	0.91±0.03	0.91±0.03
RE ($mL \cdot kg^{-1} \cdot km^{-1}$)	PEG+CHO	Male	209.5±11.3	202.4±11.3	201.4±10.7	202.9±10.1	206.6±11.0
		Female	213.3±6.8	204.2±5.4	203.6±7.7	203.1±8.5	201.7±11.1
	PEG+KME	Male	207.6±6.6	204.1±8.0	203.7±12.4	206.8±9.7	208.2±7.7
		Female	213.5±14.4	205.3±9.8	200.9±8.6	201.5±9.9	201.2±11.6
	VAP+CHO	Male	204.7±8.9	199.0±9.3	195.6±8.7	197.5±6.9	201.0±8.1
		Female	208.7±7.9	201.5±9.1	194.3±8.7	194.8±11.2	196.8±11.5
	VAP+KME	Male	207.0±11.9	199.8±10.7	197.3±11.5	199.0±10.1	200.2±8.3
		Female	208.2±8.9	199.9±6.4	195.3±8.3	196.5±9.2	196.6±10.7
RPE (AU)	PEG+CHO	Male	7.0±0.7	8.6±1.4	10.1±1.6	11.6±1.6	13.3±1.5
		Female	7.1±1.1	8.6±2.1	10.3±2.6	12.0±3.3	14.4±4.0
	PEG+KME	Male	7.6±1.3	8.9±.4	10.6±1.6	11.9±1.5	13.7±1.3
		Female	7.1±1.2	8.9±2.0	10.6±2.6	12.8±3.1	14.9±3.9
	VAP+CHO	Male	6.8±0.4	8.4±1.0	10.0±1.4	11.7±1.7	13.1±1.9
		Female	7.5±1.8	8.6±2.1	10.4±2.3	12.1±2.9	13.6±3.3
	VAP+KME	Male	7.3±0.7	8.5±0.8	10.3±1.4	11.7±1.3	13.2±1.3
		Female	7.0±1.1	8.5±1.8	10.1±2.5	11.9±2.9	13.9±3.8

Data are presented as mean±SD. Male, n=10; Female, n=8.

Supplementary Table 5. $\dot{V}O_2$ and percentage $\dot{V}O_{2peak}$ for male and female participants at each submaximal stage during each condition.

	Condition	Group	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	PEG+CHO	Male	34.9±1.9	37.1±2.1	40.3±2.2	44.0±2.2	48.2±2.6
		Female	32.0±1.0	34.1±0.9	37.3±1.4	40.6±1.7	43.7±2.4
	PEG+KME	Male	34.6±1.1	37.4±1.5	40.8±2.5	44.8±2.1	48.6±1.8
		Female	32.0±2.2	34.2±1.6	36.8±1.6	40.3±2.0	43.6±2.5
	VAP+CHO	Male	34.1±1.5	36.5±1.7	39.1±1.7	42.8±1.5	46.9±1.9
		Female	31.3±1.2	33.6±1.5	35.6±1.6	39.0±2.3	42.6±.5
	VAP+KME	Male	34.5±2.0	36.6±1.9	39.5±2.3	43.1±2.2	46.7±1.9
		Female	31.2±1.3	33.3±1.1	35.8±1.5	39.3±1.8	42.6±2.3
$\%\dot{V}O_{2peak}$	PEG+CHO	Male	54.3±3.4	57.7±4.1	62.7±4.4	68.4±4.7	75.0±5.9
		Female	60.5±5.7	64.4±6.5	70.6±6.8	76.8±7.5	82.6±8.3
	PEG+KME	Male	53.7±3.4	58.1±4.7	63.3±6.4	69.6±6.5	75.5±6.8
		Female	61.3±4.5	65.5±4.1	70.5±4.9	77.2±6.4	83.5±7.0
	VAP+CHO	Male	52.0±3.4	55.6±3.3	59.6±3.3	65.2±3.7	71.5±4.7
		Female	58.0±5.8	62.2±6.4	65.9±6.7	72.1±7.2	78.9±8.2
	VAP+KME	Male	52.6±4.4	55.8±4.4	60.2±4.8	65.7±4.6	71.2±4.8
		Female	58.7±4.9	62.8±5.4	67.3±5.0	73.9±6.0	80.1±6.7

Data are presented as mean±SD. Male, n=10; Female, n=8.

Supplementary Table 6. HR and percentage of HR_{peak} for male and female participants at each submaximal stage during each condition.

	Condition	Group	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
HR (bpm)	PEG+CHO	Male	118±9	128±10	137±9	148±9	157±8
		Female	134±5	147±3	156±3	168±3	177±4
	PEG+KME	Male	123±10	134±11	141±12	151±12	160±10
		Female	139±9	151±7	158±5	169±4	176±5
	VAP+CHO	Male	121±7	129±9	135±8	146±9	155±8
		Female	135±11	144±10	154±8	166±6	174±6
	VAP+KME	Male	123±11	132±11	138±11	147±11	156±10
		Female	138±9	148±5	155±5	166±4	175±5
$\%HR_{peak}$	PEG+CHO	Male	64.2±5.0	69.6±5.3	74.3±4.7	80.2±4.7	85.4±4.0
		Female	69.6±3.5	76.4±2.9	81.3±3.2	87.2±3.2	92.2±3.2
	PEG+KME	Male	66.7±4.9	72.7±5.6	76.4±5.7	81.9±5.1	86.7±4.7
		Female	73.4±3.6	79.8±3.1	83.2±2.0	88.9±2.3	92.7±2.6
	VAP+CHO	Male	65.8±4.1	70.2±4.4	73.8±4.5	79.5±4.6	84.4±4.0
		Female	70.0±5.1	74.9±4.5	79.9±3.7	86.0±2.5	90.4±2.9
	VAP+KME	Male	67.0±5.2	71.7±4.9	74.8±4.9	80.1±4.9	84.7±4.5
		Female	72.1±3.8	77.4±1.9	81.3±2.4	87.1±1.9	91.6±2.3

Data are presented as mean±SD. Male, n=10; Female, n=8.