american physiological society
JOURNAL OF APPLIED PHYSIOLOGY

J Appl Physiol 140: 1373–1388, 2026.
First published April 11, 2026; doi:10.1152/japplphysiol.01072.2025

RESEARCH ARTICLE

Ketone monoester reduces blood glucose, exogenous CHO oxidation, and oxidation efficiency in trained male cyclists when fed 120 g/h of CHO during exercise

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Abstract

We examined the effects of ketone monoester (KME) and carbohydrate (CHO) coingestion on exogenous CHO oxidation (via U-^{13}C-enriched glucose-fructose drinks), metabolomic responses, and exercise capacity. In a randomized crossover design (after 36 h of CHO loading and pre-exercise meal of 12 and 2 g·kg^{-1}, respectively), eight trained male cyclists ($\dot{V}O_{2max}$; 66 ± 7 mL·kg^{-1}·min^{-1}) ingested 0 g·h^{-1} (PLA), 120 g·h^{-1} CHO (CHO), or 120 g·h^{-1} CHO + 75-g ketone monoester (CHO + KME) during 3 h of cycling at power outputs corresponding to 95% of lactate threshold (LT) followed by exercise to exhaustion at 150% LT. Mean blood glucose concentrations during exercise were different between all pairwise comparisons ($P < 0.05$) such that CHO > CHO + KME > PLA (4.9 ± 0.3, 4.4 ± 0.2, 3.7 ± 0.4 mmol·L^{-1}, respectively). Mean exogenous CHO oxidation (1.35 ± 0.15 vs. 1.50 ± 0.16 g·min^{-1}, $P < 0.01$) and oxidation efficiency was lower in CHO + KME (67 ± 7%) compared with CHO (75 ± 6%, $P < 0.01$). Exercise capacity was greater ($P < 0.05$) in CHO (349 ± 189 s) and CHO + KME (319 ± 225 s) compared with PLA (75 ± 105 s), though no differences were evident between CHO and CHO + KME ($P > 0.05$). Ketone monoester ingestion increased abundance of metabolites associated with carbohydrate metabolism (glucaric acid) and protein turnover (3-methylhistidine). We conclude that ketone monoester ingestion does not enhance exercise capacity and reduces blood glucose concentrations, exogenous CHO oxidation, and oxidation efficiency when compared with CHO alone.

NEW & NOTEWORTHY To promote exercise performance, endurance athletes frequently consume CHO during exercise at ingestion rates of 90–120 g·h^{-1}. Here, we report for the first time that coingestion of a ketone monoester before and during prolonged cycling reduces blood glucose concentrations, exogenous CHO oxidation, and oxidation efficiency compared with CHO alone (120 g·h^{-1}; 1:0.8 ratio of maltodextrin and fructose). Ketone monoester ingestion also increased plasma abundance of metabolites associated with carbohydrate metabolism (glucaric acid) and protein turnover (3-methylhistidine).

fructose; ketones; maltodextrin; metabolomics; stable isotopes

INTRODUCTION

Since the introduction of the muscle biopsy technique in the late 1960s (1), the importance of muscle glycogen for augmenting exercise capacity and performance in endurance events has been well documented. In addition to high endogenous carbohydrate (CHO) availability, consumption of CHO during exercise (typically via gels, drinks or bars) is also ergogenic to exercise performance (2, 3), an effect likely mediated by liver glycogen sparing (4), maintenance of plasma glucose and whole body CHO oxidation rates (5), and/or via direct effects on the central nervous system (6). Accordingly, contemporary nutritional guidelines for competitive endurance events (>90 min in duration) typically recommend sufficient CHO loading (e.g., 10–12 g/kg body mass for 1–2 days) to ensure elevated muscle and liver glycogen stores, as well as CHO intake during exercise at rates of 90–120 g/h (7–9).

In addition to CHO, the use of oral ketone monoesters as a potential fuel source during exercise has received increased research focus in the past decade. Indeed, in contrast to nutritional ketosis (requiring days of CHO restriction), it is now accepted that oral ingestion of ketone monoesters represents a safe and tolerable strategy to acutely increase circulating β-hydroxybutyrate (βHB) to levels > 2 mmol·L^{-1} within 30 min of ingestion (10), even when consumed in the presence of high CHO availability (11). Nonetheless, although initial research demonstrated that ketone monoesters reduced muscle glycogen utilization during exercise and improved exercise performance (12), findings since have proved equivocal.

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Submitted 11 November 2025 / Revised 16 December 2025 / Accepted 1 April 2026

Indeed, numerous independent laboratories have subsequently reported no metabolic or ergogenic effects of ketone monoester ingestion, as evidenced by no alterations to muscle fuel selection in terms of muscle glycogen utilization and intramuscular triglyceride utilization (11), or improvements in exercise performance (11, 13). Moreover, in some instances, ketone monoesters have impaired endurance exercise performance (14, 15).

Our understanding of the practical application of ketone monoesters during exercise is further limited by the lack of studies that have been undertaken in conditions representative of high CHO availability, i.e., glycogen-loaded, pre-exercise CHO-rich meal and consumption of CHO during exercise. This is especially the case in relation to the latter, where in the studies conducted to date (11–13, 15–18), no researchers have yet investigated a CHO ingestion dose >60 $g \cdot h^{-1}$. The interaction of ketone monoesters and CHO coingestion during exercise is of particular interest when considering that acute ketone monoester ingestion reduces blood glucose concentration during exercise (11, 15, 16, 19, 20), thus potentially hindering the availability of the ingested CHO for subsequent oxidation. Indeed, although previous research demonstrated that coingestion of ketone monoester did not suppress exogenous CHO oxidation during 90 min of treadmill load carriage exercise (albeit with an ingestion dose of only 30 g of glucose per hour) (15), more recent data have demonstrated that post-exercise ketone and CHO coingestion (1 $g \cdot kg^{-1} \cdot h^{-1}$ of sucrose for 4 h) reduced blood glucose by >1 $mmol \cdot L^{-1}$ and increased the retention of the ingested CHO (21). Such data suggest that in conditions where larger boluses of CHO are ingested over longer durations (i.e., 90–120 $g \cdot h^{-1}$ of dual source CHO blends during several hours of exercise), ketone monoester ingestion may reduce the availability and subsequent oxidation of the CHO that has been ingested, metabolic conditions that, in turn, may impair endurance performance.

With this in mind, the primary aim of the present study was to evaluate the effects of oral ketone monoester ingestion on rates of exogenous CHO oxidation during exercise. To this end, trained male cyclists completed a 3 h steady-state submaximal cycling protocol (followed by an exercise capacity test) during which they consumed: *1*) 0 $g \cdot h^{-1}$ CHO (PLA), 120 $g \cdot h^{-1}$ CHO only (CHO), or 120 $g \cdot h^{-1}$ CHO with 75-g ketone monoester coingestion (CHO + KME). Importantly, all three experimental trials were commenced in conditions of high pre-exercise CHO availability, as achieved with a 36-h CHO loading protocol of 12 $g \cdot kg^{-1}$ and a pre-exercise meal of 2 $g \cdot kg^{-1}$ CHO. To assess the impacts of ketone monoester on exogenous CHO oxidation, CHO was consumed in fluid form (formulated in a 1:0.8 ratio of maltodextrin to fructose), and drinks were enriched with both ^{13}C-glucose and ^{13}C-fructose during preparation. We hypothesized that ketone monoester ingestion would reduce circulating blood glucose concentrations, exogenous CHO oxidation, and impair exercise capacity when compared with CHO alone.

METHODS

Ethical Approval

All participants gave written informed consent before participation in the study, after all the experimental protocols and potential risks had been explained. The study was approved by the Ethics Committee of Liverpool John Moores University (Ethics No.: 23/SPS/072) and conformed to the Declaration of Helsinki (except for registration in a trials registry).

Participants

Eight endurance-trained amateur male cyclists (means ± SD: age, 30 ± 9 yr; body mass, 78.3 ± 8.1 kg; height, 182 ± 6 cm) voluntarily participated in the study. Mean maximal oxygen consumption ($\dot{V}O_{2max}$), peak power output (PPO), and power output at lactate threshold (LT) were 65.9 ± 6.7 $mL \cdot kg^{-1} \cdot min^{-1}$ (range; 54.4–75.1 $mL \cdot kg^{-1} \cdot min^{-1}$), 394 ± 22 W, and 203 ± 22 W, respectively. Participants were classified as trained (Tier 2) in accordance with the framework criteria specified by McKay et al. (22). None of the participants had any history of neurological or musculoskeletal disease, nor were they taking any pharmacological treatments or undertaking a ketogenic diet in the lead up to or during the course of the testing period.

Experimental Overview

In a randomized, single-blind, crossover design (separated by >6 days), participants completed a prolonged endurance-based cycling exercise protocol comprised of 180-min steady-state submaximal exercise (undertaken at power output corresponding to 95% lactate threshold) followed by an exercise capacity test to exhaustion (undertaken at power output corresponding to 150% of lactate threshold) on four separate occasions. The initial trial was a full familiarization trial (FAM) to the exercise and feeding protocols (whereby only placebo drinks were consumed during exercise). In the following three randomized trials, participants either ingested 120 $g \cdot h^{-1}$ CHO + 75 g KME (CHO + KME), 120 $g \cdot h^{-1}$ CHO + KME placebo (CHO), or 0 $g \cdot h^{-1}$ CHO + KME placebo (PLA), all in fluid form during the exercise protocol. Each trial was commenced following a glycogen depletion protocol, followed by a 36 h CHO loading protocol (12 $g \cdot kg^{-1}$) and 2 h after a high CHO pre-exercise meal (2 $g \cdot kg^{-1}$). An overview of the experimental design and feeding protocols is displayed in Fig. 1.

Preliminary Testing

At least 7 days before experimental trials, all participants performed a two-part incremental cycle test on an ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine lactate threshold (LT), maximal oxygen consumption ($\dot{V}O_{2max}$), and peak power output (PPO) as previously described (23). The first part of the incremental cycling test was commenced at 100 W and increased by 25 W at the end of each 4-min stage. A fingertip capillary blood sample was obtained during the final 30 s of each stage to determine blood lactate concentrations (Biosen C-Line; EKF Diagnostics, Cardiff, UK). The LT and lactate turnpoint (LTP) were determined by the first and second sustained increases in blood lactate above baseline values (24). Participants began the second part of the test at the corresponding intensity to the penultimate stage completed during the first part of the test, and intensity increased by 25 W each minute until volitional exhaustion

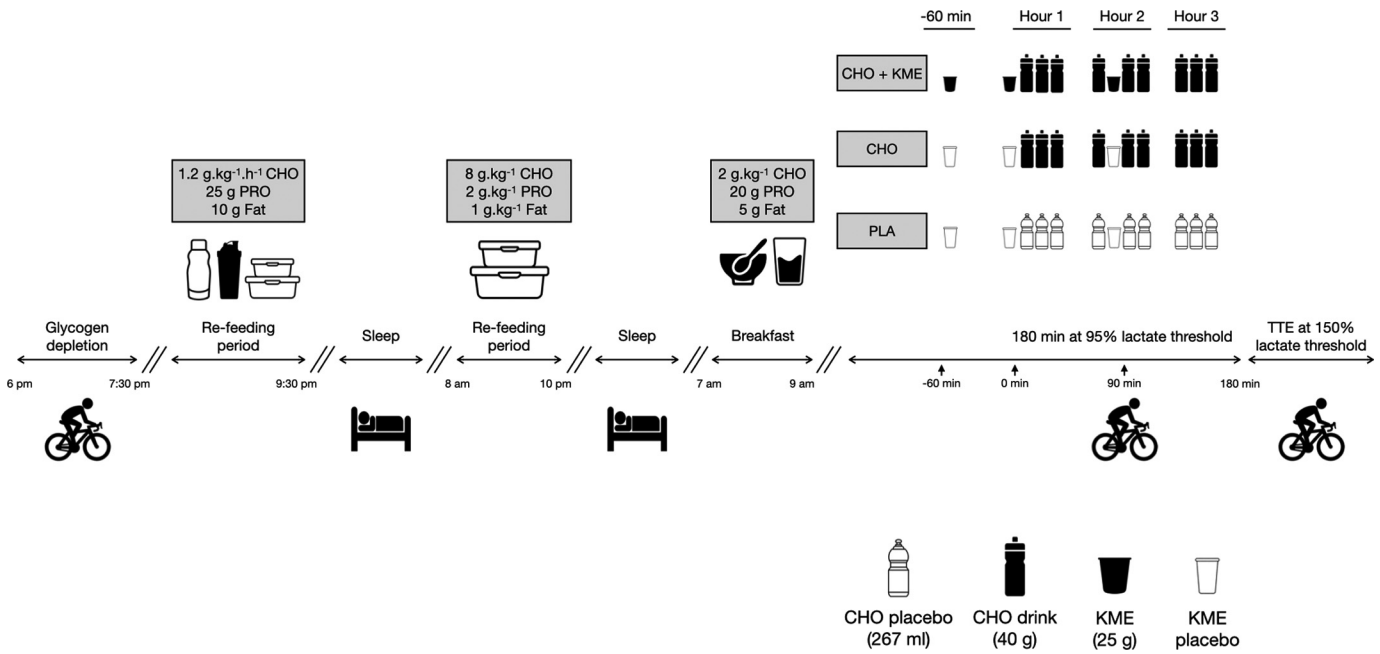


Figure 1. Schematic overview of the experimental protocol used in each trial. On the evening of *day 1*, participants completed a glycogen-depleting cycling protocol, followed by a high-carbohydrate (CHO) diet for 3 h, before sleep. During *day 2*, participants consumed a high CHO diet. On *day 3*, participants consumed a high CHO pre-exercise meal before undertaking 180-min steady-state submaximal exercise whereby they consumed 120 g·h^{-1} CHO and 75-g ketone monoester (KME) (CHO + KME), 120 g·h^{-1} carbohydrate and KME placebo (CHO), or CHO placebo and KME placebo (PLA), followed immediately by a time to exhaustion (TTE) exercise capacity test. $N = 8$ participants. Arrow (↑) denotes KME or KME placebo ingestion at −60, 0, and 90 min.

was reached. The test end time and power output at the point of exhaustion were used to calculate PPO using the following equation (25):

$$\text{PPO} = \text{W}_{\text{final}} + (t/60) \times \text{PI}$$

W_{final} is the power output of the final completed stage of the test, t is time spent in the final uncompleted stage (seconds), 60 is the duration of each stage (seconds), and PI is the increment increase in power output between stages (W). $\dot{\text{V}}\text{O}_{2\text{max}}$ was assessed using an online gas analysis system (Moxus Modular Metabolic system; AEI Technologies, Illinois) and was determined as the highest average oxygen consumption ($\dot{\text{V}}\text{O}_2$) recorded over a 30 s period. The same gas analyzer was used during all subsequent experimental trials.

Familiarization and Experimental Protocols

At least 7 days following preliminary testing, and at least 7 days before the main experimental trials, participants completed a full familiarization to the trial. This served to familiarize participants to the pre-exercise conditions, exercise protocols, feeding strategies, and to confirm the participant's ability to complete the prescribed exercise intensity during the 180-min steady-state cycle, and to also provide baseline data for the exercise capacity test. During the submaximal cycling exercise protocol, participants refrained from CHO and KME intake, therefore replicating the same nutritional conditions as the PLA trial (without venous blood samples and expired breath samples being obtained).

Day 1: Glycogen Depletion Protocol

At 36 h before each trial, participants attended the laboratory in the evening to perform a 90-min intermittent glycogen-depleting cycling protocol (on *day 1*), as previously described within our laboratory (26). This pattern of intermittent exercise was chosen in an attempt to induce glycogen depletion in both type I and II muscle fibers. The pattern of exercise and total time to exhaustion from the glycogen-depleting ride were recorded during their first trial and subsequently replicated in each of the remaining trials. Immediately, post 1 h and 2 h after the glycogen-depletion exercise, participants consumed high CHO fluids/snacks to facilitate the goal of CHO loading (1.2 g·kg^{-1}·h^{-1}).

Day 2: CHO Loading

Over the following day, participants were provided with prepackaged food and drinks as part of a standardized high CHO diet (8 g·kg^{-1} CHO, 2 g·kg^{-1} PRO, 1 g·kg^{-1} FAT) to consume throughout the whole day (i.e., breakfast cereals, pasta, rice, confectionary products). Participants also refrained from any alcohol consumption and any form of exercise, while water was allowed ad libitum. A high CHO breakfast (2 g·kg^{-1} CHO, ∼20 g PRO, ∼5 g FAT) was also provided to participants for consumption the following morning, 3 h before the commencement of exercise, in accordance with contemporary sports nutrition guidelines for endurance exercise (7). Foods with a high natural abundance of ^{13}C (e.g., corn and sugar cane) were avoided in the standardized diet and breakfast, to minimize the background shift from glycogen stores during exercise. In addition, the prior bout of glycogen-depleting exercise also served to reduce endogenous ^{13}C stored in glycogen. The success of this approach was verified by minimal background shifts in breath $^{13}\text{CO}_2$ enrichment during completion of the placebo trial (see Fig. 6A). In

addition, there was no difference in resting breath $^{13}CO_2$ enrichment (i.e., the time point corresponding to 0 min in Fig. 4*A*) between all trials ($P = 0.14$).

Day 3: Experimental Trials

On the morning of the experimental trials, participants attended the laboratory at ~09:00, having already consumed the prepackaged standardized breakfast previously provided to them. First, an indwelling cannula (Nexiva Closed IV catheter 22 G; BD Biosciences, Worthing, UK) was inserted into the antecubital vein in the anterior crease of the forearm and a baseline resting blood sample was drawn and subsequently flushed with ~5 mL of sterile saline (BD Posiflush) and this procedure was repeated after each blood draw to ensure the cannula remained sterile and patent. A fingertip capillary blood sample was also collected to determine blood βHB concentrations (Freestyle Optimum Neo, Abbott, Berkshire, UK), blood glucose, and lactate (Biosen C-Line; EKF Diagnostics, Cardiff, UK). Immediately after this, participants were provided with their first 25-g KME or KME placebo beverage. One hour later, another resting blood sample was taken before resting expired breath samples were collected in duplicate directly from the mixing chamber on the gas analyzer (Moxus, Modular Metabolic system; AEI Technologies, Illinois), into evacuated 10-mL exetainer tubes (Labco, High Wycombe, UK). This was to determine the ^{13}C-to-^{12}C ratio in CO_2 at rest. Participants were then provided with their second 25-g KME or KME placebo beverage, as well as a serving of 40-g CHO or CHO placebo (provided at 20-min intervals from this point). Participants then commenced the 180-min cycling protocol at 95% of LT (193 ± 20 W). The choice of this relative exercise intensity was due to its suggestion as an appropriate method of matching metabolic stress between participants when compared with exercising at a percentage of $\dot{V}O_{2max}$ (27). Expired gas was collected for 5-min periods at 30-min intervals to calculate whole body substrate utilization. During the final 1 min of each period, expired gas was collected into the evacuated exetainer tubes from the mixing chamber on the gas analyzer, to determine the ^{13}C-to-^{12}C ratio in CO_2. Blood samples were collected at 30-min intervals during exercise, along with heart rate (Polar H10; Polar, Kempele, Finland) and ratings of perceived exertion (RPE) (28). At 30-min intervals, gastrointestinal (GI) symptoms were recorded (nausea, regurgitation, fullness, cramps, gas, and urge to defecate) using a visual analog scale (whereby 0 = no discomfort and 10 = very severe discomfort) (29). At each time point, the sum of scores was combined for each symptom, to provide a maximum score of 60. At 90-min during the 180-min cycle, participants were provided with their third and final 25-g KME or KME placebo beverage. Immediately following the 180-min cycle, participants commenced the exercise capacity test to volitional exhaustion at 150% of LT (305 ± 32 W), defined as an inability to maintain a cadence >60 rpm. No music was played, and no verbal encouragement from the researcher was given during this test and the only information available to participants was the fixed power output (W). No performance results were given to the participant until completion of all the experimental trials. Each experimental trial was performed under normal laboratory conditions (21°C and 50% humidity) at the same time of day (10:00 ± 1 h), using the same electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) and the same automated online gas analyzer (Moxus Modular Metabolic System, AEI Technologies, Illinois). During all exercise trials, a floor-standing fan was used to cool participants to minimize thermal stress. Each experimental trial was separated by >6 days and participants were asked to continue with their habitual training schedule during this period.

Carbohydrate Feeding

During exercise in the CHO + KME and CHO experimental trials, participants consumed CHO at a rate of 120 $g \cdot h^{-1}$ using multiple-transportable carbohydrates in a 1:0.8 ratio of maltodextrin to fructose in fluid form (Beta Fuel powder, Science in Sport, Blackburn, UK). Carbohydrate was ingested immediately before exercise began, and at 20-min intervals during exercise in equal amounts of 40 g per serving, with the last carbohydrate drink being provided at 160 min of the 180-min ride. Carbohydrate drinks were mixed with 800 mL of water (per 120 g CHO), resulting in a 15% CHO solution and a fluid intake of 800 $mL \cdot h^{-1}$. This absolute volume of fluid intake is commensurate with the habitual fluid intake of elite endurance athletes (30). In CHO + KME and CHO trials, the carbohydrate drinks were equivalently enriched with 100 mg of stable isotope tracer, comprising 56 mg of U-^{13}C-glucose and 44 mg of U-^{13}C-fructose (CK isotopes, Ibstock, UK) per 120 g of CHO. This ensured there were equivalent proportions of tracer to match their tracee and the ratio of maltodextrin (glucose) and fructose. During FAM and PLA exercise trials, a taste-matched, noncaloric carbohydrate placebo (Science in Sport, Blackburn, UK) was consumed across the trial. Participants consumed an equivalent amount of fluid (267 mL at each time point) to ensure the pattern of ingestion and total fluid intake was matched across all trials.

Ketone Monoester Feeding

Participants ingested a total of 75-g KME [>96% (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate] during the CHO + KME or a viscosity and taste-matched placebo beverage during FAM, PLA, and CHO trials. In all trials, the KME or KME placebo was delivered in 3 × 25 g equal boluses and ingested at *1*) 60 min before (−60), *2*) immediately before (0 min) the commencement of the 180-min cycle, and *3*) at minute 90 of the 180-min cycle, aiming to establish physiological ketosis throughout the duration of the steady-state exercise bout and exercise capacity test. The KME was purchased from TdeltaS (△G Tactical Ketones, △G, TdeltaS, Thame, Oxfordshire, UK) and was combined with flavor drops (FlavDrops, Myprotein, Altrincham, UK), noncaloric sucralose sweetener (Bulk, Essex, UK), and water. The KME placebo was prepared by dissolving 1 mM bitter sucrose octaacetate (Sigma-Aldrich, Bornem, Belgium) with flavor drops, noncaloric sucralose sweetener, and water. Upon completion of each trial, all participants confirmed no differences in taste between treatments. When expressed relative to each participant's body mass, the relative dose of KME equated to

3 × repeated doses of 323 ± 35 mg·kg^{-1}. BM and a total dose of 968 ± 105 mg·kg^{-1}.

Blood Sampling and Analysis

Venous blood samples were collected into Vacutainers contained K2EDTA (BD Biosciences, UK) and stored on ice until centrifugation at 1,500 *g* for 10 min at 4°C. Following centrifugation, plasma samples were aliquoted and stored at −80°C for subsequent analysis. Samples were later analyzed for glycerol and nonesterified fatty acids (NEFAs) with commercially available enzymatic spectrophotometric assays (RX Daytona + Analyzer; Randox, UK) as per the manufacturer's instructions (analytical coefficient of variation, CV, <2% and <5%, respectively). Note that plasma NEFA and glycerol data are representative of $n = 7$ only due to a catheter fault with one participant. Capillary fingertip blood samples were obtained at each time point and immediately analyzed for blood glucose and blood lactate (Biosen C-Line; EKF Diagnostics, Cardiff, UK) and β-hydroxybutyrate (βHB) (Abbott freestyle optimum neo, Abbott, UK) (analytical CVs all <3%).

Estimates of Whole Body Substrate Oxidation and Energy Expenditure

Rates of whole body CHO and fat oxidation (g·min^{-1}) were calculated from expired gas collected at 30-min intervals throughout each 180-min steady-state submaximal cycle during each experimental trial (31). Substrate utilization data during PLA are missing at 150-min time point for one participant and at 180-min for three participants due to volitional fatigue.

$^{13}C/^{12}C$ Analysis of Breath CO_2

Isotopic enrichment of breath samples was determined by gas chromatography isotope ratio mass spectrometry (Europa Scientific 20-20; Iso-Analytical Ltd., Crewe, UK). ^{13}C enrichment from expired breath samples is expressed as $\delta^{13}C$ ‰ versus Pee Dee Belemnite (PDB). Exogenous carbohydrate oxidation was calculated using the following formula:

$$\text{Exogenous CHO oxidation } (\text{g} \cdot \text{min}^{-1}) =$$
$$\dot{V}co_2 \times (\delta\text{Exp} - \delta\text{Expbkg})/(\delta\text{Ing} - \delta\text{Expbkg})/\text{k}$$

$\dot{V}CO_2$ is carbon dioxide production, δExp is the ^{13}C enrichment of the expired CO_2, δIng is the ^{13}C enrichment of the ingested carbohydrate, δExp_{bkg} is the ^{13}C enrichment of expired CO_2 at rest, and k is the $\dot{V}CO_2$ with the oxidation of 1 g of glucose (0.7467 L·CO_2·g^{-1}).

^{1}H-NMR Metabolomics

Sample preparation.

Previously aliquoted plasma was thawed and diluted at 50% (vol/vol) with NMR buffer (20% $2H_2O$: 80% $1H_2O$ with 200 mM sodium phosphate buffer pH 7.4 and 2.4 mM azide) before centrifugation for 5 min at 21,500 *g* and 4°C. Six hundred microliters of the centrifuged sample was pipetted into 5 mm (outer diameter) glass SampleJet NMR tubes (Bruker, Coventry, UK).

^{1}H-NMR set-up and acquisition.

All spectra were acquired on a Bruker 700 MHz Avance III HD spectrometer equipped with a TCI cryoprobe and chilled "SampleJet" autosampler (Bruker, Coventry, UK). Standard vendor pulse sequences were applied to collect 1-D 1H-NMR spectra (cpmgpre1d). A Carr–Purcell–Meiboom–Gill (CPMG)-edited pulse sequence was used to attenuate signals from macromolecules present (proteins, etc.). Serum spectra were collected at 37°C with 32 transients for optimal sensitivity, with all other parameters constant. Parameter sets along with raw and processed spectra were deposited in the EMBL European Bioinformatics Institute (EBI) repository MetaboLights (MTBLS6260).

Spectral processing and quality control.

All spectra were preprocessed at the spectrometer by Fourier transformation, phase correction, and baseline correction using standard vendor routines (apk0.noe) and referenced against anomeric glucose signal at 5.24 ppm. Spectra were subject to quality control criteria as recommended by the Metabolomics Standards Initiative (MSI) (32, 33), comprising an appraisal of baseline, line-width, residual water signal width, phase, and signal-to-noise. Spectra were bucketed according to peak boundaries defined with each bucket, the sum of the integral for that region divided by the region width.

Metabolite annotation and identification.

Metabolites were annotated via metabolite recognition software Chenomx (Chenomx v 8.2, Chenomx Ltd, Edmonton, Alberta, Canada), and the respective buckets were annotated before statistical analysis. Metabolite identities were confirmed (where possible) by comparison with an in-house metabolite library. While the entirety of the serum metabolome is measured by ^{1}H-NMR spectroscopy, adherence to strict quality control, identification, and reporting guidelines significantly reduced the number of metabolites presented compared with unvalidated mass spectrometry approaches.

Statistical analysis of metabolites.

Metabolomics analyses were performed using the statistical software Rstudio (v. 4.5.1) with scripts provided by the Computational Biology Facility (CBF) at the University of Liverpool (UK). Data normalization was conducted in MetaboAnalyst (v. 6.0), using probabilistic quotient normalization (PQN). Due to the robustness of PQN in the analysis of complex biofluids (34), all spectra underwent PQN normalization to the nonketone monoester groups to avoid data skewing to additional molecules observed in CHO + KME group (35). Samples from −60 min pre-exercise, 0 min pre-exercise, and 180 min post-exercise across CHO + KME, CHO, and PLA conditions were analyzed via linear mixed-effects model. *P* values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) method, with adjusted P values < 0.05 considered significant. Significant effects were further examined using Tukey's post hoc pairwise comparisons between conditions and time points. Boxplots were generated using the ggplot2 package, and heatmaps were produced using pheatmap (Rstudio).

Statistical Analysis

All statistical analysis (except for metabolomics analysis) were performed using SPSS Statistics version 27 (IBM). A

linear mixed-effects, repeated-measures model was used to analyze differences over time and between conditions for substrate utilization (rates of whole body and exogenous CHO oxidation, fat oxidation, RER), cardiorespiratory measures (HR and $\dot{V}O_2$), RPE, blood, and plasma metabolite concentrations. Where a significant main effect was observed, pairwise comparisons were analyzed by using post hoc least significance difference (LSD) tests to locate specific differences. Substrate utilization, HR, RPE, and $\dot{V}O_2$ were analyzed from 30 to 180 min, blood βHB from −60 to 180 min, and other blood parameters (glucose, NEFA, glycerol, and lactate) from 0 to 180 min. Paired *t* tests were also used to analyze differences in mean hourly exogenous CHO oxidation, oxidation efficiency, and peak exogenous CHO oxidation. Effect sizes for exogenous CHO oxidation and oxidation efficiency are presented as Hedges *g* (with 0.2, 0.5, and >0.8 representing a small, moderate, and large effect, respectively), and 95% confidence intervals (CIs) for differences are also presented. Friedman's ANOVA was used to analyze differences in total exercise duration and exercise capacity, and where a significant main effect was observed, pairwise comparisons were conducted using the Wilcoxon signed-rank test, and the median difference was estimated using the Hodges–Lehmann method and accompanied by 95% confidence intervals (CIs). Effect sizes for total exercise duration and exercise capacity were calculated from the Wilcoxon signed-rank tests, using $r = Z/\sqrt{N}$, where Z is the test statistic, and N is the number of paired comparisons. Effect sizes are interpreted as representing a small, moderate, and large effect, for *r* values of 0.1, 0.3, and >0.5, respectively. Cumulative and peak scores of gastrointestinal discomfort were analyzed using Friedman's ANOVA. All data in text and figures are presented as means ± SD, with *P* values ≤ 0.05 indicating statistical significance.

RESULTS

Effects of Ketone Monoester Ingestion on Blood βHB Concentrations before and throughout Exercise

In accordance with the ingestion of ketone monoester before and during exercise, blood βHB concentrations were significantly higher at all time points (across 0 to 180 min) compared with both CHO and PLA (treatment effect, $P < 0.01$; time effect, $P < 0.01$) (Fig. 2*A*).

Effects of Ketone Monoester Ingestion and CHO Availability on Physiological and Perceptual Responses to Exercise

Heart rate and absolute oxygen uptake (see Table 1) increased during exercise (time effect, $P < 0.01$ for both variables) although no differences were apparent between trials ($P = 0.07$, $P = 0.90$, respectively). RPE increased during exercise (time effect, $P < 0.01$; treatment effect, $P < 0.01$; Table 1) with greater RPE reported during PLA than CHO ($P < 0.01$) and CHO + KME ($P = 0.01$).

Effects of Ketone Monoester Ingestion and CHO Availability on Whole Body CHO and Lipid Metabolism

Blood glucose concentrations were different between trial conditions (treatment effect, $P < 0.01$; time effect, $P = 0.48$), with concentrations greater in CHO versus CHO + KME ($P < 0.01$) and PLA ($P < 0.01$). Blood glucose concentrations were also greater during CHO + KME than PLA ($P < 0.01$) (Fig. 2*B*).

Plasma NEFA concentrations were different between trial conditions (treatment effect, $P < 0.01$; time effect, $P < 0.01$) (Fig. 2*C*) such that NEFA concentrations were greater during PLA than CHO ($P < 0.01$) and CHO + KME ($P < 0.01$). In addition, NEFA concentration during exercise were reduced in CHO + KME versus CHO ($P < 0.01$). Similarly, plasma glycerol concentrations were different between trial conditions (treatment effect, $P < 0.01$; time effect, $P < 0.01$) (Fig. 2*D*). Glycerol concentrations were greater during PLA than both CHO and CHO + KME (both $P < 0.01$), yet were reduced in CHO + KME versus CHO ($P = 0.02$). There was no difference detected in blood lactate concentrations between trials (time effect $P = 0.38$; treatment effect $P = 0.09$; Fig. 2*E*).

Respiratory exchange ratio (RER) decreased throughout exercise (time effect, $P < 0.01$), with significantly lower RER evident in the PLA trial (0.85 ± 0.05) compared with CHO and CHO + KME trials (trial effect, $P < 0.01$; interaction effect, $P < 0.01$). There was no difference in RER (0.89 ± 0.03 vs. 0.89 ± 0.02, $P = 0.76$) during exercise between CHO and CHO + KME trials, respectively (Fig. 3*A*). Rates of whole body CHO oxidation progressively decreased during exercise (time effect, $P < 0.01$), with significantly lower mean rates of CHO oxidation evident in the PLA trial (1.82 ± 0.52 g·min^{-1}) compared with CHO and CHO + KME trials (trial effect, $P < 0.01$; interaction effect, $P < 0.01$) (Fig. 3*B*). However, there was no difference in rates of mean whole body CHO oxidation between CHO + KME and CHO (2.36 ± 0.29 vs. 2.27 ± 0.32 g·min^{-1}, $P = 0.52$) (Fig. 3*B*). Whole body rates of fat oxidation (Fig. 3*C*) progressively increased during exercise (time effect, $P < 0.001$), whereby during the PLA trial (0.75 ± 0.24 g·min^{-1}), mean rates of whole body fat oxidation were significantly greater than CHO and CHO + KME trials (trial effect, $P < 0.01$; interaction effect, $P < 0.01$). There was no difference in mean rates of fat oxidation during exercise between CHO + KME and CHO (0.53 ± 0.11 vs. 0.56 ± 0.15 g·min^{-1}, $P = 0.65$) (Fig. 3*C*).

The contribution of both whole body CHO and fat oxidation toward energy expenditure during exercise is presented in Fig. 3, *D*–*F*.

Effects of Ketone Monoester Ingestion and CHO Availability on Exogenous CHO Oxidation and Oxidation Efficiency

Mean breath $^{13}CO_2$ enrichment (treatment effect, $P < 0.01$; time effect, $P < 0.01$) was greater during exercise in CHO compared with CHO + KME ($P = 0.03$) and PLA ($P < 0.01$). Mean breath $^{13}CO_2$ enrichment was also greater during CHO + KME compared with PLA ($P < 0.01$) (Fig. 4*A*).

Accordingly, when assessed over the entire 3 h of exercise, there was a significantly lower mean rate of exogenous CHO oxidation (1.35 ± 0.15 vs. 1.50 ± 0.16 g·min^{-1}; time effect, $P < 0.01$; treatment effect, $P < 0.01$, mean difference, −0.15, 95% CI [−0.22 to −0.07 g·min^{-1}], Hedges $g = 1.48$, large) (Fig. 4*B*) and oxidation efficiency (67 ± 7 vs. 75 ± 6%, mean difference −8, $P < 0.01$, 95% CI [−12 to −4%], Hedges $g = 1.46$, large) in

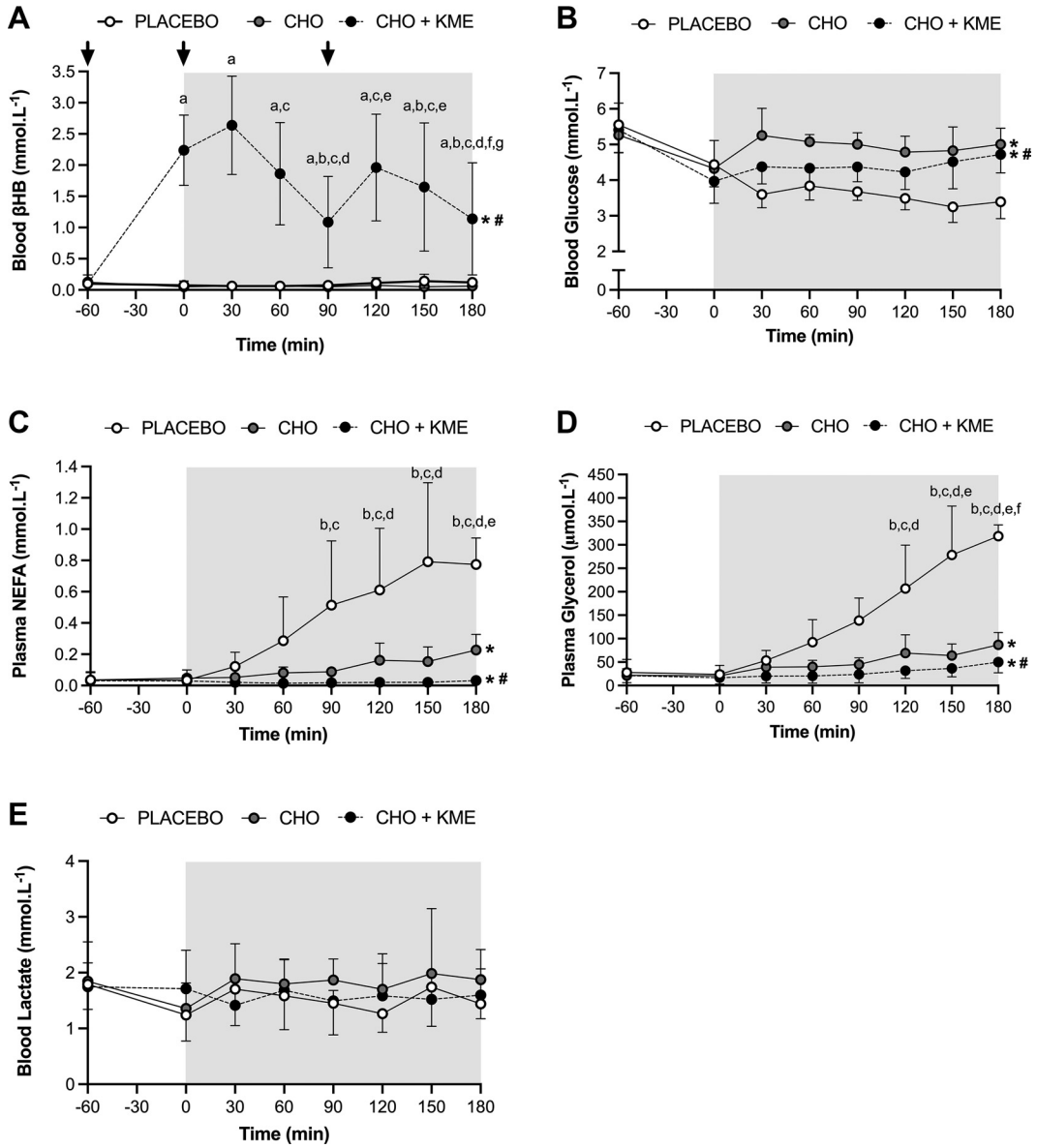


Figure 2. Blood βHB (*A*), blood glucose (*B*), plasma nonesterified fatty acids (NEFAs; *C*), plasma glycerol (*D*), and blood lactate (*E*) during the experimental protocol. [a,b,c,d,e,f,g]$P < 0.05$ vs. −60 (a), 0 (b), 30 (c), 60 (d), 90 (e), 120 (f), and 150 min (g), respectively. *Significant difference from PLA, $P < 0.05$, #significant difference from CHO, $P < 0.05$. Data are means ± SD. Statistical differences in blood metabolite concentrations were assessed using a linear mixed-effects, repeated measures model. Arrow (⬇) denotes 25-g ketone monoester ingestion. Gray shaded area represents period of exercise. Blood βHB, glucose, and lactate, $n = 8$. Plasma NEFA and plasma glycerol, $n = 7$.

the CHO + KME trial versus the CHO trial, respectively (Fig. 4*C*).

When assessed over the first hour of exercise, there was a significantly lower rate of exogenous CHO oxidation (0.80 ± 0.13 vs. 0.96 ± 0.19 g·min^{-1}; $P = 0.03$, mean difference −0.16, 95% CI [−0.30 to −0.02 g·min^{-1}], Hedges $g = 0.82$, large) and oxidation efficiency (40 ± 7 vs. 48 ± 9%, $P = 0.033$, mean difference −8, 95% CI [−15 to −1%], Hedges $g = 0.83$, large) in the CHO + KME trial versus the CHO trial, respectively.

When assessed over the second hour of exercise, there was a significantly lower rate of exogenous CHO oxidation (1.53 ± 0.13 vs. 1.70 ± 0.14 g·min^{-1}; $P < 0.01$, mean difference −0.17, 95% CI [−0.26 to −0.07 g·min^{-1}], Hedges $g = 1.36$, large) (Fig. 4*E*) and oxidation efficiency (77 ± 8 vs. 85 ± 7%, $P < 0.01$, mean difference −8, 95% CI [−13 to −4%], Hedges $g = 1.37$, large) in the CHO + KME trial versus the CHO trial, respectively.

When assessed over the third hour of exercise, differences in rates of exogenous CHO oxidation (1.70 ± 0.13 vs. 1.80 ± 0.14 g·min^{-1}; $P = 0.06$, mean difference −0.10, 95% CI [−0.21 to 0.01 g·min^{-1}], Hedges $g = 0.67$, moderate) (Fig. 4*F*) and oxidation efficiency (85 ± 6 vs. 90 ± 7%, $P = 0.066$, mean difference −5, 95% CI [−10 to 0%] Hedges $g = 0.68$, moderate) trended toward significance between trials and lower values were observed in the CHO + KME trial versus the CHO trial, respectively.

When exogenous CHO oxidation was assessed over both *hours 2* and *3* (inclusive), there was a significantly lower rate of exogenous CHO oxidation (1.62 ± 0.14 vs. 1.75 ± 0.13 g·min^{-1}; $P < 0.01$, mean difference −0.13, 95% CI [−0.21 to −0.05

Table 1. *Heart rate, RPE, and $\dot{V}O_2$ during exercise*

	Time, min					
	30	60	90	120	150	180
Heart rate, beats/min						
PLA	133 ± 5	134 ± 8	136 ± 9[a]	139 ± 9[a,b]	146 ± 4[a,b,c,d]	152 ± 3[a,b,c,d,e]
CHO	132 ± 7	136 ± 8	140 ± 9[a]	141 ± 8[a,b]	143 ± 9[a,b,c,d]	145 ± 11[a,b,c,d,e]
CHO + KME	138 ± 10	139 ± 10	140 ± 9[a]	143 ± 9[a,b]	146 ± 9[a,b,c,d]	149 ± 10[a,b,c,d,e]
RPE, AU						
PLA	11 ± 2	13 ± 1	14 ± 1	15 ± 1[a,b]	16 ± 1[a,b,c]	17 ± 2[a,b,c]
CHO*	10 ± 2	12 ± 2	13 ± 2	13 ± 2[a,b]	14 ± 2[a,b,c]	15 ± 2[a,b,c]
CHO + KME*	11 ± 1	12 ± 1	13 ± 1	14 ± 2[a,b]	14 ± 2[a,b,c]	15 ± 2[a,b,c]
$\dot{V}O_2$, L·min^{-1}						
PLA	2.92 ± 0.40	2.91 ± 0.37	2.98 ± 0.43[a,b]	2.97 ± 0.42[a]	3.06 ± 0.43[a,b,c,d]	3.08 ± 0.26[a,b,c,d]
CHO	2.90 ± 0.41	2.94 ± 0.43	2.97 ± 0.40[a,b]	2.97 ± 0.38[a]	2.99 ± 0.40[a,b,c,d]	3.01 ± 0.41[a,b,c,d]
CHO + KME	2.93 ± 0.42	2.96 ± 0.42	3.00 ± 0.46[a,b]	2.96 ± 0.43[a]	3.00 ± 0.41[a,b,c,d]	3.03 ± 0.43[a,b,c,d]

[a,b,c,d,e]$P < 0.05$ vs. 30 (a), 60 (b), 90 (c), 120 (d), and 150 min (e), respectively. *Significant difference from PLA, $P < 0.05$. Data are means ± SD, $n = 8$. Statistical differences were assessed using a linear mixed-effects repeated measures model.

g·min^{-1}], Hedges $g = 1.18$, large) and oxidation efficiency (81 ± 7 vs. 88 ± 7%, $P < 0.01$, mean difference −7, 95% CI [−10 to −3%], Hedges $g = 1.28$, large) in the CHO + KME trial versus the CHO trial, respectively.

Peak rates of exogenous CHO oxidation were greater during CHO (1.84 ± 0.12 g·min^{-1}) compared with CHO + KME (1.76 ± 0.15 g·min^{-1}; $P < 0.01$, mean difference 0.08, 95% CI [0.03 to 0.14 g·min^{-1}], Hedges $g = 1.19$, large) (Fig. 4*D*).

Effects of Ketone Monoester Ingestion and CHO Availability on Gastrointestinal Discomfort

Mean cumulative scores for each gastrointestinal symptom were less than moderate (all trials <4 out of a possible 60, except for two trials), with no significant differences between trials (treatment effect for nausea, $P = 0.202$; regurgitation, $P = 0.717$; stomach fullness, $P > 0.99$; abdominal cramps, $P = 0.368$; gas or flatulence, $P = 0.627$; urge to defecate, $P = 0.368$). There were no differences detected in peak scores for each symptom between trials (treatment effect for nausea, $P = 0.061$; regurgitation, $P = 0.717$; stomach fullness, $P = 0.368$; abdominal cramps, $P = 0.368$; gas or flatulence, $P = 0.807$; urge to defecate, $P = 0.368$).

Effects of Ketone Monoester Ingestion and CHO Availability on Total Exercise Duration and Exercise Capacity

Three participants reached volitional fatigue before completion of the 3-h steady-state exercise period during the PLA trial. Therefore, total exercise duration (duration of steady-state exercise + exercise capacity test) was greater in both CHO (03:05:48 ± 00:03:09 h:min:s) and CHO + KME (03:05:19 ± 00:03:45 h:min:s) compared with PLA (02:48:32 ± 00:18:59 h:min:s, median difference, 0:16:22 h:min:s, $Z = -2.38$, $P = 0.02$, 95% CI [0:02:40 to 0:31:53 h:min:s], $r = 0.84$, large; median difference, 0:15:43 h:min:s, $Z = -2.10$, $P = 0.04$, 95% CI [0:02:08 to 0:32:25 h:min:s], $r = 0.74$, large, respectively). However, there was no difference in total exercise duration between CHO + KME and CHO trials (median difference, 0:00:17 h:min:s, $Z = -0.70$, $P = 0.48$, 95% CI [−0:01:32 to 0:03:00 h:min:s], $r = 0.25$, small) (Fig. 5*A*).

When assessing exercise capacity (time to exhaustion at 150% LT) immediately following the steady-state exercise, both CHO (349 ± 189 s) and CHO + KME (319 ± 225 s) significantly enhanced exercise capacity compared with PLA (75 ± 105 s, median difference, 283 s, $Z = -2.38$, $P = 0.02$, 95% CI [111 to 423 s], r = 0.84, large; median difference, 217 s, $Z = -2.10$, $P = 0.04$, 95% CI [33 to 495 s], $r = 0.74$, large, respectively). However, there was no significant difference in exercise capacity between CHO and CHO + KME trials (median difference 17 s, $Z = -0.70$, $P = 0.48$, 95% CI [−92 to 180 s], $r = 0.25$, small) (Fig. 5*B*). Furthermore, there was no significant effect of trial order on exercise capacity ($P = 0.17$).

Metabolomic Analysis of Plasma

In total, 51 metabolites were quantified by NMR. We used a linear mixed-effects model to test the effects of trial, time point, and their interaction on each metabolite, and *P* values were adjusted for multiple comparisons using false discovery rate (FDR) correction. Ten significant interactions (trial × time) were present; 2-hydroxybutyric acid, 3-hydroxybutyric acid, acetic acid, acetoacetic acid, acetone, D-glucose, glucaric acid, glycylproline, 3-methyhistidine, and N-alpha-acetyl-L-lysine (Fig. 6*A*). When compared with baseline, ketone monoester ingestion (CHO + KME) increased the abundance of six plasma metabolites at time points 0 (i.e., 60 min after the initial bolus of ketone ingestion) and 180 min (i.e., after 3 h of exercise and after a further two boluses of ketone monoester ingestion) including 2-hydroxybutyric acid, 3-hydroxybutyric acid, acetone, acetoacetic acid, glucaric acid, and 3-methylhistidine. These changes were not observed in PLA or CHO (Fig. 6*A*).

DISCUSSION

Confirming our hypothesis, we report for the first time that coingestion of a ketone monoester (75 g) with CHO intake at a rate of 120 g·h^{-1} during exercise (1:0.8 ratio of maltodextrin and fructose) reduces blood glucose concentrations, exogenous CHO oxidation, and oxidation efficiency when compared with CHO alone. As expected, CHO ingestion improved exercise capacity compared with placebo conditions, though exercise capacity was neither enhanced nor impaired by the coingestion of ketone monoester. When considered with previous literature (11, 13–15), these data provide further evidence that ketone monoester ingestion before and during exercise does not offer a metabolic or ergogenic advantage for prolonged endurance performance above and

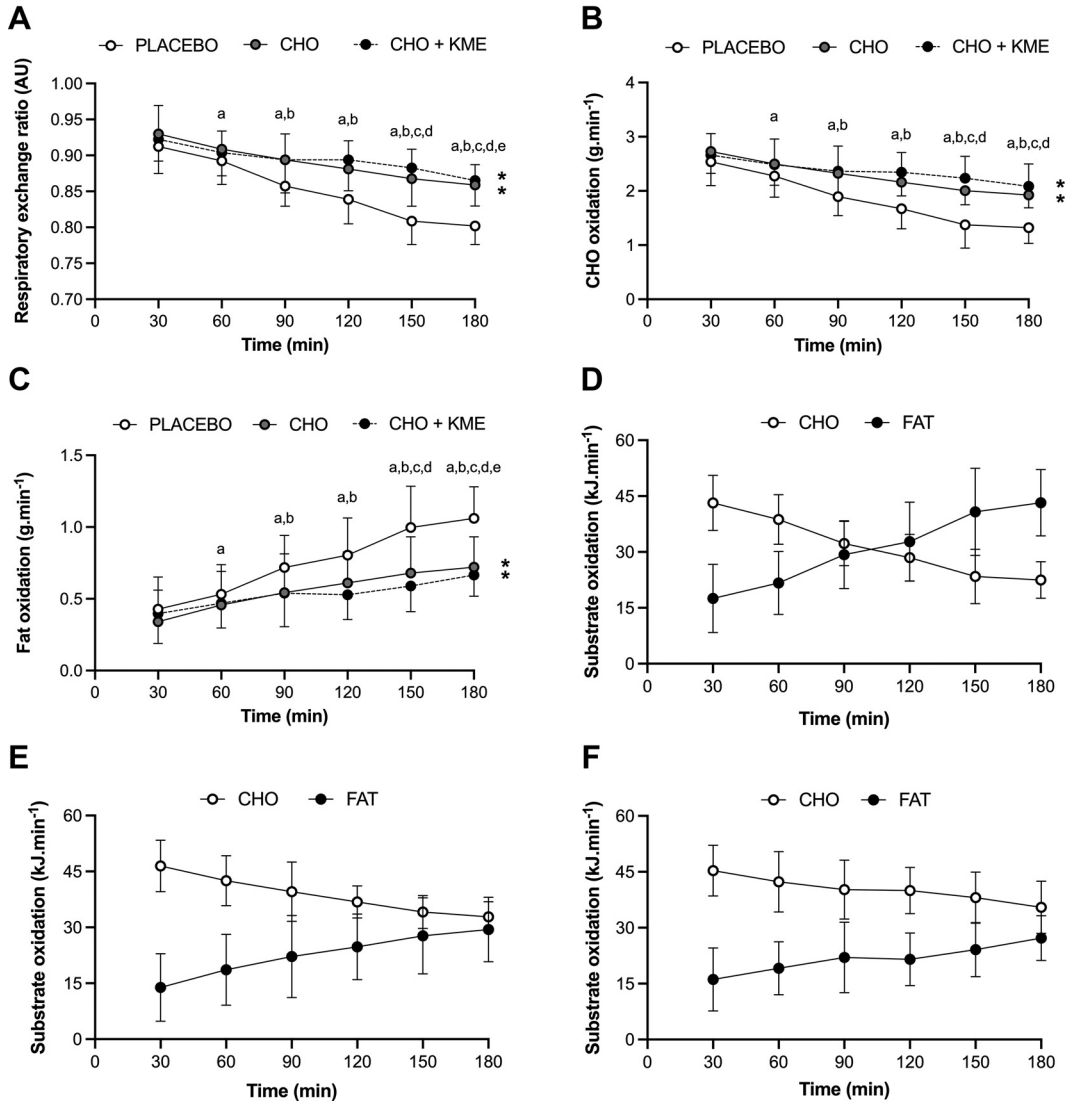


Figure 3. Respiratory exchange ratio (RER; *A*), whole body CHO oxidation (*B*), and fat oxidation rates (*C*) during exercise. Rates of energy provision from CHO and fat oxidation during the PLA (*D*), CHO (*E*), and CHO + KME (*F*) trials. $^{a,b,c,d,e}P < 0.05$ vs. 30 (a), 60 (b), 90 (c), 120 (d), and 150 min (e), respectively. *Significant difference from PLA, $P < 0.05$. Data are means ± SD, $n = 8$. Statistical differences were assessed using a linear mixed-effects, repeated measures model.

beyond that of conditions already representative of high CHO availability.

To address our aims, we studied a cohort of trained male cyclists who completed an exercise protocol that has previously been utilized within our laboratory (i.e., 3 h of steady-state cycling at 95% of lactate threshold followed by an exercise capacity test to exhaustion at 150% of lactate threshold) (8, 23). Importantly, participants commenced exercise in conditions representative of best practice "race day" nutrition (i.e., glycogen-loaded and after a pre-exercise CHO-rich meal) as well as high CHO consumption during exercise. To achieve ketosis from the onset and throughout exercise, we chose to feed a repeated absolute 25-g bolus of a commercially available ketone monoester at 60 min before exercise, at the beginning of exercise and after 90 min of exercise. This approach proved successful in maintaining elevated circulating βHB concentrations within the range of 1–3 mmol·L^{-1} (see Fig. 2*A*). Consistent with previous reports (11, 12, 36), we also observed an apparent antilipolytic effect of ketone monoesters, such that circulating NEFA and glycerol concentrations were reduced during exercise when compared with CHO alone (see Fig. 2, *C* and *D*). Such effects are likely mediated by direct inhibitory effects of βHB on the regulation of lipolysis within the adipocyte, via G-protein-coupled receptor 109 A (GPR109A)-mediated signaling (11, 37). In addition, untargeted metabolomics also demonstrated marked differences in the plasma metabolome before and during exercise (see Fig. 6), with an increased abundance of metabolites associated with ketone body formation, carbohydrate, and protein metabolism. When taken together, such data collectively demonstrate that the dose and frequency of ketone monoester ingestion used here was sufficient to induce distinct metabolic differences between trials.

In extending these well-documented metabolic effects of ketone monoesters, the novel aspect of the present

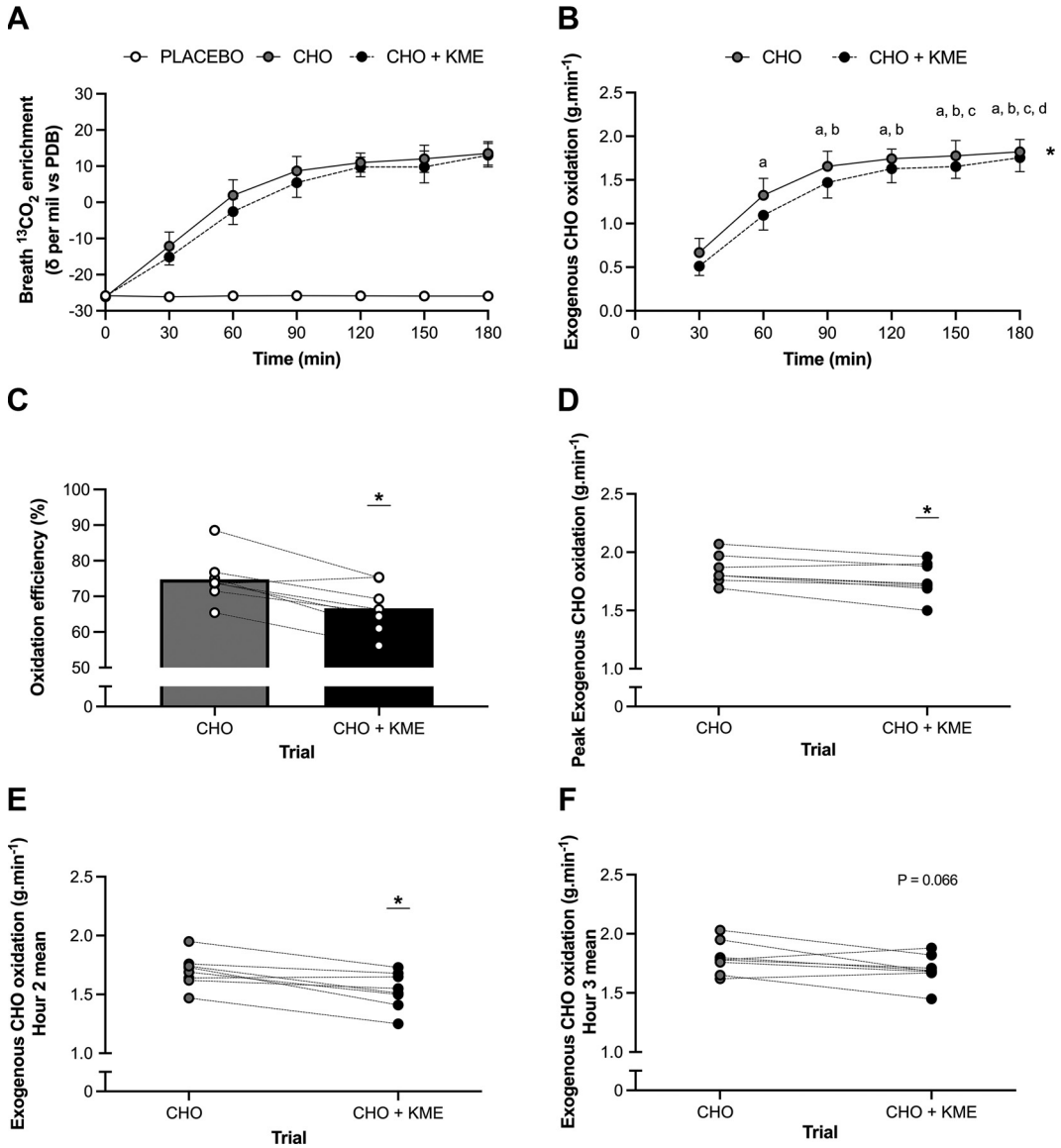


Figure 4. Breath $^{13}CO_2$ enrichment (*A*) and exogenous CHO oxidation (*B*) during 180 min of exercise during the PLA, CHO, and CHO + KME trials. Mean CHO oxidation efficiency (*C*), peak exogenous CHO oxidation (*D*), mean exogenous CHO oxidation during *hour 2* (*E*), and *hour 3* (*F*) of exercise. $^{a,b,c,d}P <$ 0.05 vs. 30 (a), 60 (b), 90 (c), and 120 min (d), respectively. *Significant difference from CHO trial, $P < 0.05$. Data are means ± SD, $n = 8$. Statistical differences in exogenous CHO oxidation (*B*) were assessed using a linear mixed-effects model; paired *t* test was used to assess mean oxidation efficiency (*C*), peak exogenous CHO oxidation (*D*), mean exogenous CHO oxidation during hour 2 (*E*) and hour 3 (*F*).

study was the observation that ketone monoester coingestion reduced absolute rates of exogenous CHO oxidation during exercise, as was the case for a comparison of mean rates of oxidation across the full 3 h (1.35 ± 0.15 vs. 1.50 ± 0.16 g·min^{-1}), as well as the first (0.80 ± 0.13 vs. 0.96 ± 0.19 g·min^{-1}), second (1.54 ± 0.16 vs. 1.70 ± 0.14 g·min^{-1}), and third (1.70 ± 0.13 vs. 1.80 ± 0.14 g·min^{-1}) hour of exercise. In addition, participants' peak rates of exogenous CHO oxidation were also reduced with ketone monoester ingestion (1.76 ± 0.15 vs. 1.84 ± 0.12 g·min^{-1}). As such, ketone monoester reduced exogenous CHO oxidation efficiency (67 ± 7 vs. 75 ± 7%) and suppressed absolute rates of exogenous CHO oxidation by ∼10, ∼10, and ∼6 g·h^{-1} in *hours 1, 2,* and *3*, respectively. In our previous research that also evaluated exogenous CHO oxidation rates with CHO ingestion rates of 120 g·h^{-1} (8), we observed a within-participant variation (standard deviation) for exogenous carbohydrate oxidation of 4.2 g·h^{-1}. Accordingly, we consider the smallest worthwhile difference in exogenous carbohydrate oxidation to be 5 g·h^{-1}, and on this basis, the observed difference in exogenous CHO oxidation (∼9 g·h^{-1}) reported here is both statistically significant and physiologically relevant.

To the authors' knowledge, there is only one previous study investigating rates of exogenous CHO oxidation during exercise when coingested with a ketone monoester (15). In that study, recreationally active men completed a 90-min steady-state treadmill run at 54% $\dot{V}O_{2peak}$ (while wearing a weighted vest), and CHO was consumed during exercise at a lower absolute rate (i.e., 30 g·h^{-1} of glucose). Despite a

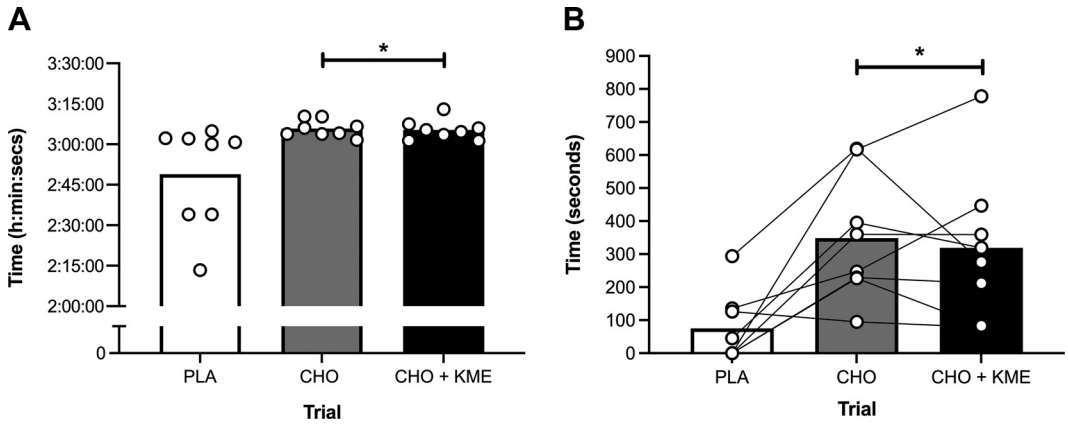


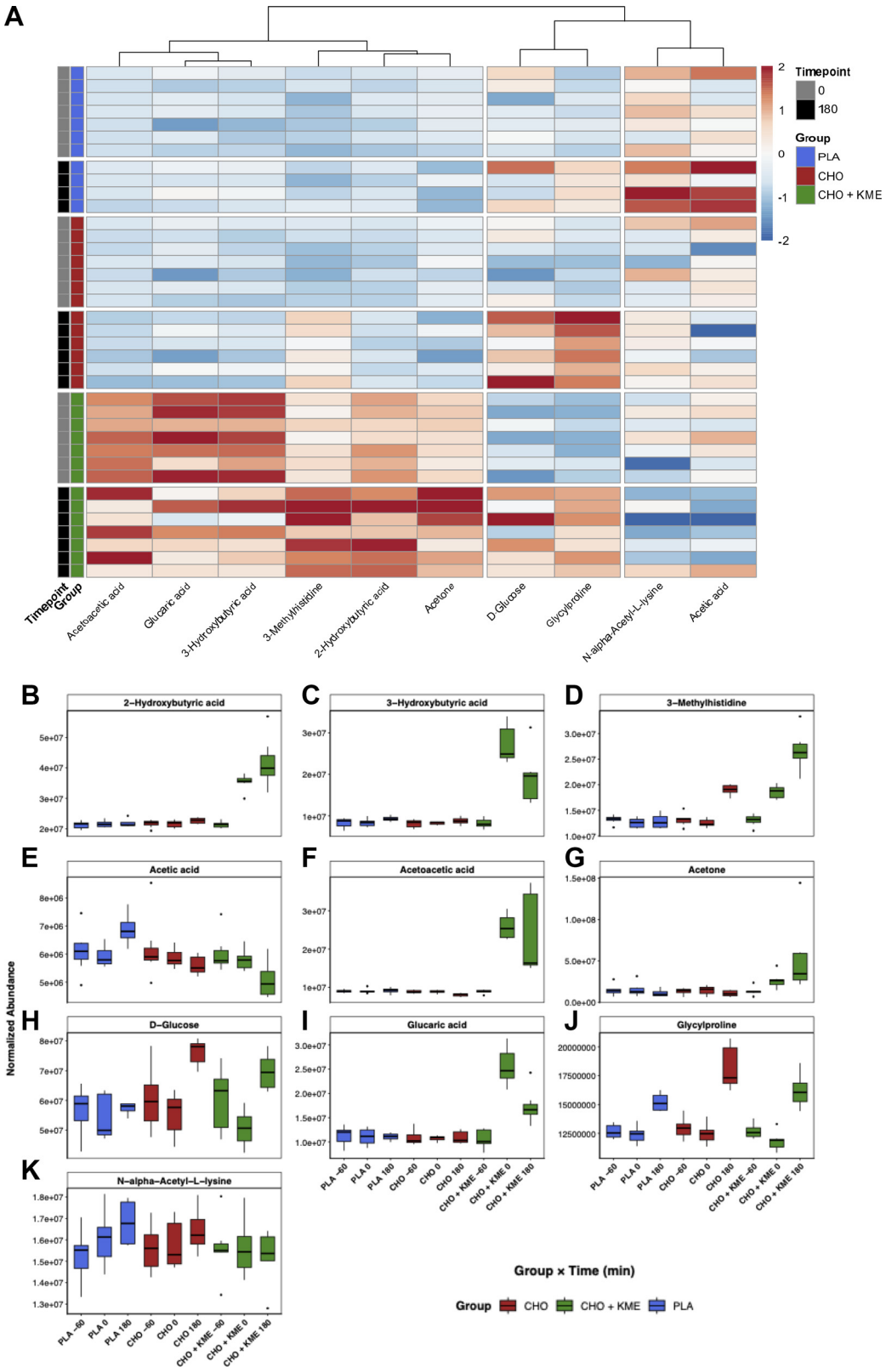
Figure 5. Total exercise duration (h:min:s) (*A*) and exercise capacity time (s) (*B*) during the PLA, CHO, and CHO + KME trials. *Significant difference from PLA, $P < 0.05$. $N = 8$, statistical differences were assessed using a Friedman's ANOVA, with pairwise comparisons using the Wilcoxon Signed-rank test.

reduced rate of glucose appearance (Ra) and disappearance (Rd) during exercise, exogenous CHO oxidation was not affected by ketone monoester ingestion and comparable exogenous oxidation rates (~0.5 g·min^{-1}) were observed between trials. Interestingly, these researchers also reported lower blood glucose concentrations during exercise and suggested that the reduced glucose Ra associated with ketone monoester ingestion was likely due to reduced hepatic glycogenolysis, given that plasma ^{13}C enrichments were not different between CHO and CHO + KME trials. Indeed, the finding that acute ketone monoester ingestion (i.e., a bolus consumed across 1–3 h, as opposed to chronic ingestion over days, weeks, or months) reduces blood glucose concentration is well reported within the literature, as evident in both resting (21, 38, 39) and exercise conditions (11, 12, 15). In such contexts, it is thought that acute ketosis reduces both hepatic gluconeogenesis (due to a reduction in precursors, e.g., glycerol) and liver glycogenolysis (40, 41).

Consistent with these researchers, we also observed reduced blood glucose concentrations during exercise (see Fig. 2*B*). However, in the absence of assessments of plasma ^{13}C enrichments, it is difficult to ascertain if the reduced glucose concentrations observed here is due to reduced gluconeogenesis (as mediated by reduced glycerol availability, see Fig. 2*D*), liver glycogenolysis and/or reduced (or delayed) gastric emptying, digestion and absorption of the ingested CHO such that overall hepatic glucose output is subsequently reduced. Nonetheless, given the higher absolute dose of CHO ingestion used here (i.e., 120 g·h^{-1} for 3 h equating to a total dose of 200 g of maltodextrin and 160 g fructose), it is plausible to suggest that it is a delayed Ra of the ingested CHO that may, primarily, contribute to the reduced rates of exogenous CHO oxidation reported here. Indeed, the largest absolute differences in exogenous CHO oxidation between the CHO and CHO + KME trials (i.e., 0.23 g·min^{-1}) coincided with the period (i.e., the first and second hour of exercise) where the largest differences in both circulating βHB (i.e., 2.5 mmol·L^{-1}) and glucose (i.e., ~1 mmol·L^{-1}) were also evident. In contrast, in the final minutes of the third hour of exercise, blood glucose and exogenous CHO oxidation were beginning to transition to comparable absolute concentrations and rates of oxidation (see Figs. 2*B* and 4*B*, respectively). Support for this hypothesis is also provided by the data presented by Monteyne et al. (42), whereby ketone monoester ingestion (albeit in participants with type 2 diabetes) reduced postprandial glucose concentrations by 18% in the initial 2-h period after a mixed meal tolerance test (comprising 75-g CHO). However, no differences were apparent in total glucose Ra during the entire 4 h postprandial period owing to a greater exogenous glucose Ra from *hours 2–4* in the ketone trial. More recently, Clarke et al. (21) also reported in endurance-trained males that postexercise coingestion of ketone monoester (0.29 g·kg^{-1}·h^{-1}) and sucrose ingestion (1 g·kg^{-1}·h^{-1} for 4 h) increased the retention of the ingested CHO by ~14 g and reduced glycemia (~1 mmol·L^{-1}) compared with CHO alone. In this instance, it was proposed that enhanced splanchnic extraction and glycogen synthesis (as opposed to reduced gastric emptying) is more likely to have mediated the reduced blood glucose concentrations associated with ketone monoester ingestion. Notwithstanding the obvious differences between the previously cited studies (i.e., resting conditions and postexercise recovery) and the present experimental design (i.e., during exercise), these data collectively suggest that coingestion of a ketone monoester with high doses of CHO may ultimately reduce the circulating availability of the ingested CHO, thereby reducing the absolute rate of exogenous CHO oxidation. Further mechanistic studies are now required to directly test this hypothesis.

In accordance with previous research from our laboratory adopting similar participant training status, an identical exercise protocol and absolute CHO dose (8), we also report that exercise capacity was significantly greater in both the CHO and CHO + KME trials when compared with the placebo trial (see Fig. 5*B*). Such ergogenic effects of CHO feeding are likely due to the maintenance of blood glucose concentrations (see Fig. 2*B*) and whole body rates of CHO oxidation (see Fig. 3*B*), such that the target exercise intensity could be sustained for greater duration. Indeed, in the absence of CHO ingestion, the "crossover point" during exercise (i.e., the point at which fat becomes the predominant fuel source) occurred after 100 min of cycling (see Fig. 3*D*). In contrast, the consumption of CHO at a rate of 120 g·h^{-1} prevented the occurrence of this shift in substrate utilization such that CHO dependency was still maintained. Such data also agree with our previous observations (8) and provide further evidence that an absolute dose of 120 g·h^{-1} appears sufficient to maintain CHO dependency during exercise, at least during the exercise protocol studied here.

Importantly, the magnitude of improvement in exercise capacity observed here and previously (i.e., several minutes) is of practical significance given that competitive cycling races (e.g., stages within the cycling Grand Tours) can be won or lost with such time durations. Despite the observed differences in exogenous CHO oxidation between CHO and CHO + KME, these differences did not translate to any enhancement or impairment in exercise capacity, likely due to the comparable



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blood glucose concentrations and whole body rates of CHO oxidation at the point of commencing the exercise capacity test (see Figs. 2*B* and 3B). Such data therefore demonstrate that CHO availability was comparable and not limiting to exercise capacity at this time. Nonetheless, the present data extend previous research (11, 13, 18, 43, 44) that ketone ester ingestion before and during exercise is unlikely to offer any ergogenic benefits for prolonged endurance performance. In this regard, our data provide further support for the recent statement from the Union Cycliste Internationale (UCI) recommending that professional cyclists do not consume ketone monoester supplements as "part of their nutritional plans" due to the lack of supporting evidence (45).

Untargeted metabolomics provides new insight into how ketone and CHO coingestion regulate metabolism during exercise. As expected, ketone monoester ingestion increased the abundance of several metabolites derived from ketone bodies (2-hydroxybutyric acid, 3-hydroxybutyric acid, acetoacetic acid, and acetone), an effect apparent within 60 min of ingestion that persisted throughout the 3-h exercise protocol (Fig. 6, *B, C, F,* and *G*). However, we also provide the first report to demonstrate that ketone monoester ingestion increased the plasma abundance of both glucaric acid and 3-methylhistidine within 60 min of ketone monoester ingestion and throughout exercise (see Fig. 6, *D* and *I*). Notably, 3-methylhistidine is a key component of the myofibrillar proteins actin and myosin (46) and is released during their degradation, without being subsequently reincorporated into protein. As such, the appearance of 3-methylhistidine in urinary excretion (47) and plasma (48) is thought to be a biomarker of myofibrillar proteolysis. It is possible that this apparent response of muscle protein degradation may be a compensatory mechanism to provide amino acids for gluconeogenesis and maintain blood glucose concentrations, though it is noted that we did not detect increased abundance of such amino acids with ketone monoester ingestion. Alternatively, ketone monoesters may act as signaling molecules regulating muscle protein turnover and remodeling processes (e.g., autophagy, mitophagy), rather than directly accelerating protein breakdown (49, 50). Our observation of increased glucaric acid abundance upon ketone monoester ingestion and throughout exercise (see Fig. 6*I*) is also suggestive of increased flux through the hepatic glucuronic pathway (51). The flux through this pathway may reflect a diversion of glucose-6-phosphate from glycolysis and gluconeogenesis such that it is now oxidized to glucaric acid (52). Although the physiological relevance of this pathway remains to be fully elucidated, enhanced hepatic production of glucaric acid and thus increased oxidative glucose disposal, may partially explain the reduced circulating glucose concentrations observed with ketone monoester ingestion.

Despite the novelty of the present study, several limitations should be acknowledged. Although indirect calorimetry combined with ^{13}C stable isotope methodology was used to quantify exogenous carbohydrate oxidation, we were unable to directly partition ketone body oxidation from that of other substrates. However, using a ^{13}C-labeled ketone monoester (252 $mg \cdot kg^{-1}$ body mass), Dearlove et al. (53) demonstrated that exogenous β-hydroxybutyrate (βHB) oxidation during cycling at 75% of peak power output was ~0.1 $g \cdot min^{-1}$, accounting for <3% of total energy expenditure. Accordingly, the direct contribution of βHB oxidation to whole body substrate oxidation in the present study is likely to be small. Such βHB oxidation rates (~0.1 $g \cdot min^{-1}$) would correspond to ~0.1 $L \cdot min^{-1}$ (<4%) of metabolic CO_2 production (53). In addition, βHB ingestion constitutes an exogenous acid load. Dissociation of β-hydroxybutyric acid releases H^+, which is buffered predominantly by bicarbonate according to $H^+ + HCO_3^- \rightarrow CO_2 + H_2O$ (16). As a result, ~1 mol of nonmetabolic CO_2 is generated per mol of βHB via acid-base buffering, independent of oxidative metabolism. Based on the circulating βHB concentrations observed in the present study, this buffering-related CO_2 production would be <0.01 $L \cdot min^{-1}$ (<2%) (54) and is therefore unlikely to meaningfully influence indirect calorimetry-derived estimates of substrate oxidation. In addition, although we used ^{13}C glucose to trace maltodextrin, maltodextrin (as a soluble α-1,4 glucose polymer) is rapidly hydrolyzed by intestinal enzymes to free glucose before absorption. Once hydrolyzed, maltodextrin-derived glucose and the ingested ^{13}C-glucose tracer enter the same systemic pool via SGLT1/GLUT2 and follow identical oxidative pathways (55). Indeed, previous data demonstrated that $^{13}CO_2$ enrichment during exercise was comparable when a ^{13}C glucose tracer was used to trace the oxidation of both a glucose and glucose polymer beverage (~100 g of CHO) during 2 h of steady-state cycling at submaximal intensity (56). Furthermore, more recent data from our laboratory demonstrated that plasma U-^{13}C glucose enrichment reached a plateau within 30–120 min of exercise when participants ingested 60 $g \cdot h^{-1}$ of a maltodextrin solution, thereby excluding differences in appearance kinetics between the maltodextrin solution and the U-^{13}C tracer (57). Moreover, we also observed that breath $^{13}CO_2$ enrichment reached and maintained a plateau during exercise across trials (see Fig. 6*A*), and breath $^{13}CO_2$ and exogenous CHO oxidation exhibited the expected time-course and magnitude of response observed when comparable CHO doses have been studied where glucose has been used as the tracee (58, 59) and where the oxidation of naturally enriched glucose, maltodextrin, and fructose have been assessed (60–62). Although our ketone monoester placebo beverage was taste-matched, it was not isocaloric, and consequently, the total of 75 g of ketone monoester provided within the CHO + KME condition provided an additional 375 kcal compared with the CHO condition. However,

Figure 6. *A*: individual log2-fold changes of metabolites relative to baseline (−60 min) across treatment conditions and time points. Heatmap shows hierarchical clustering of metabolites based on log2-fold changes (vs. baseline, −60 min) at subsequent time points (0 and 180 min) in individuals. Each column represents a participant at a specific time point and treatment condition. Metabolite clustering was performed using Ward's method on Euclidean distances. KME = CHO + KME condition. Condition (left bar); KME, green; CHO, red; PLA, blue. Fold change relative to baseline (−60 min); red indicates an increase, and blue indicates a decrease in metabolite abundance relative to baseline. *B*–*K*: boxplots of significant group × time point interactions (FDR < 0.05). Data expressed as means ± SD, for normalized abundance of metabolite. 2-Hydroxybutyric acid (*B*); 3-hydroxybutyric acid (*C*); 3-methylhistidine (*D*); acetic acid (*E*); acetoacetic acid (*F*); acetone (*G*); D-glucose (*H*); glucaric acid (*I*); glycylproline (*J*); N-α-acetyl-L-lysine (*K*). Metabolomics analysis, $n = 7$. PLA 180 is $n = 4$ due to volitional fatigue. CHO 180 is $n = 6$ due to a catheter fault. CHO, carbohydrate; KME, ketone monoester; PLA, placebo.

given the previously reported low rates of exogenous βHB oxidation (i.e., ∼0.1 g·min^{-1}) (53), it is unlikely that this additional energy provision meaningfully influenced whole body substrate metabolism or exercise capacity. Finally, although the sample size within the present study is sufficient to detect differences in the primary outcome variable (exogenous CHO oxidation), we acknowledge that the present study is likely underpowered to detect differences in secondary outcomes, such as cardiorespiratory variables (e.g., heart rate and oxygen uptake). Indeed, recent studies incorporating larger sample sizes (n = 15 and 28, respectively) have reported that acute KME ingestion increased heart rate during submaximal cycling exercise protocols (63, 64).

In summary, we report for the first time that ketone monoester ingestion before and during prolonged cycling exercise reduces circulating blood glucose, exogenous rates of CHO oxidation, and oxidation efficiency compared with CHO ingestion alone. Although such differences in exogenous CHO oxidation did not impair exercise capacity, our data extend a growing body of literature suggesting that ketone monoesters are unlikely to offer a metabolic or ergogenic advantage for endurance performance beyond that of nutritional conditions already representative of high CHO availability. In using untargeted metabolomics, we also provide novel data by demonstrating that ketone monoester ingestion increased the abundance of metabolites associated with carbohydrate metabolism (glucaric acid) and protein turnover (3-methylhistidine). Future research should now explore the mechanisms by which ketone monoesters apparently impair the ability to oxidize carbohydrate that is ingested during exercise.

DATA AVAILABILITY

Data will be made available upon reasonable request.

GRANTS

This study was funded by a research grant from Science in Sport Limited.

DISCLOSURES

J.P.M. is a consultant for Science in Sport (SiS). SiS, Glaxosmithkline (GSK), and Lucozade Ribena Suntory (LRS) have previously funded J.P.M.'s research on CHO metabolism and exercise. J.N.P. has received an honorarium from SiS. D.J.O. has a research grant from Gencor Pacific Ltd. J.T.G. has received research funding from BBSRC, MRC, British Heart Foundation, Clasado Biosciences, Lucozade Ribena Suntory, ARLA Foods Ingredients, Cosun Nutrition Center, Innocent Drinks, and the Fruit Juice Science Center; is a scientific advisory board member to ZOE; and has completed paid consultancy for 6d Sports Nutrition, Science in Sport, The Dairy Council, PepsiCo, Violicom Medical, Tour Racing Ltd., and SVGC. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

H.J.M., J.T.G., S.O.S., J.N.P., and J.P.M. conceived and designed research; H.J.M. and N.E.C. performed experiments; H.J.M., J.A.E., M.M.P., and D.J.O. analyzed data; H.J.M., J.N.P., D.J.O., and J.P.M. interpreted results of experiments; H.J.M. and J.A.E. prepared figures; H.J.M., D.J.O., and J.P.M. drafted manuscript; H.J.M. and J.P.M. edited and revised manuscript; H.J.M., N.E.C., J.T.G., S.O.S., J.N.P., D.J.O., and J.P.M. approved final version of manuscript.

REFERENCES

1. **Bergström J**, **Hermansen L**, **Hultman E**, **Saltin B.** Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71: 140–150, 1967. doi:10.1111/j.1748-1716.1967.tb03720.x.
2. **Currell K**, **Jeukendrup AE.** Superior endurance performance with ingestion of multiple transportable carbohydrates. *Med Sci Sports Exerc* 40: 275–281, 2008. doi:10.1249/mss.0b013e31815adf19.
3. **Rowlands DS**, **Swift M**, **Ros M**, **Green JG.** Composite versus single transportable carbohydrate solution enhances race and laboratory cycling performance. *Appl Physiol Nutr Metab* 37: 425–436, 2012. doi:10.1139/h2012-013.
4. **Gonzalez JT**, **Fuchs CJ**, **Smith FE**, **Thelwall PE**, **Taylor R**, **Stevenson EJ**, **Trenell MI**, **Cermak NM**, **van Loon LJC.** Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *Am J Physiol Endocrinol Metab* 309: E1032–E1039, 2015. doi:10.1152/ajpendo.00376.2015.
5. **Coyle EF**, **Coggan AR**, **Hemmert MK**, **Ivy JL.** Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol (1985)* 61: 165–172, 1986. doi:10.1152/jappl.1986.61.1.165.
6. **Chambers ES**, **Bridge MW**, **Jones DA.** Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. *J Physiol* 587: 1779–1794, 2009. doi:10.1113/jphysiol.2008.164285.
7. **Thomas DT**, **Erdman KA**, **Burke LM.** American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Med Sci Sports Exerc* 48: 543–568, 2016 [Erratum in *Med Sci Sports Exerc* 49: 222, 2017]. doi:10.1249/MSS.0000000000000852.
8. **Hearris MA**, **Pugh JN**, **Langan-Evans C**, **Mann SJ**, **Burke L**, **Stellingwerff T**, **Gonzalez JT**, **Morton JP.** ^{13}C-glucose-fructose labeling reveals comparable exogenous CHO oxidation during exercise when consuming 120 g/h in fluid, gel, jelly chew, or coingestion. *J Appl Physiol (1985)* 132: 1394–1406, 2022. doi:10.1152/japplphysiol.00091.2022.
9. **Morton JP**, **Fell JM**, **Gonzalez JT**, **Hearris MA**, **Podlogar T**, **Pugh JN**, **Wallis GA.** From metabolism to medals: contemporary perspectives and revisiting carbohydrate guidelines for fuelling endurance athletes during exercise. *J Nutr* 156: 101442, 2026. doi:10.1016/j.tjnut.2026.101442.
10. **Soto-Mota A**, **Vansant H**, **Evans RD**, **Clarke K.** Safety and tolerability of sustained exogenous ketosis using ketone monoester drinks for 28 days in healthy adults. *Regul Toxicol Pharmacol* 109: 104506, 2019. doi:10.1016/j.yrtph.2019.104506.
11. **Poffé C**, **Ramaekers M**, **Bogaerts S**, **Hespel P.** Exogenous ketosis impacts neither performance nor muscle glycogen breakdown in prolonged endurance exercise. *J Appl Physiol (1985)* 128: 1643–1653, 2020. doi:10.1152/japplphysiol.00092.2020.
12. **Cox PJ**, **Kirk T**, **Ashmore T**, **Willerton K**, **Evans R**, **Smith A**, **Murray AJ**, **Stubbs B**, **West J**, **McLure SW**, **King MT**, **Dodd MS**, **Holloway C**, **Neubauer S**, **Drawer S**, **Veech RL**, **Griffin JL**, **Clarke K.** Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. *Cell Metab* 24: 256–268, 2016. doi:10.1016/j.cmet.2016.07.010.
13. **Evans M**, **McSwiney FT**, **Brady AJ**, **Egan B.** No benefit of ingestion of a ketone monoester supplement on 10-km running performance. *Med Sci Sports Exerc* 51: 2506–2515, 2019. doi:10.1249/MSS.0000000000002065.
14. **McCarthy DG**, **Bone J**, **Fong M**, **Pinckaers PJ**, **Bostad W**, **Richards DL**, **van Loon LJ**, **Gibala MJ.** Acute ketone monoester supplementation impairs 20-min time-trial performance in trained cyclists: a randomized, crossover trial. *Int J Sport Nutr Exerc Metab* 33: 181–188, 2023. doi:10.1123/ijsnem.2022-0255.
15. **Howard EE**, **Allen JT**, **Coleman JL**, **Small SD**, **Karl JP**, **O'Fallon KS**, **Margolis LM.** Ketone monoester plus carbohydrate supplementation does not alter exogenous and plasma glucose oxidation or metabolic clearance rate during exercise in men compared with

carbohydrate alone. *J Nutr* 153: 1696–1709, 2023. doi:10.1016/j.tjnut.2023.03.002.

16. **Poffé C**, **Ramaekers M**, **Bogaerts S**, **Hespel P.** Bicarbonate unlocks the ergogenic action of ketone monoester intake in endurance exercise. *Med Sci Sports Exerc* 53: 431–441, 2021. doi:10.1249/MSS.0000000000002467.
17. **Leckey JJ**, **Ross ML**, **Quod M**, **Hawley JA**, **Burke LM.** Ketone diester ingestion impairs time-trial performance in professional cyclists. *Front Physiol* 8: 806–806, 2017. doi:10.3389/fphys.2017.00806.
18. **Evans M**, **Egan B.** Intermittent running and cognitive performance after ketone ester ingestion. *Med Sci Sports Exerc* 50: 2330–2338, 2018. doi:10.1249/MSS.0000000000001700.
19. **Brady AJ**, **Moynagh MB**, **Devenney S**, **Egan B.** Advanced footwear technology, but not acute ingestion of a ketone monoester, improves running economy in middle- and long-distance runners. *Med Sci Sports Exerc* 57: 1559–1569, 2025. doi:10.1249/MSS.0000000000003682.
20. **Waldman HS**, **O'Neal EK**, **Barker GA**, **Witt CR**, **Lara DA**, **Huber AK**, **Forsythe VN**, **Koutnik AP**, **D'Agostino DP**, **Staiano W**, **Egan B.** A ketone monoester with carbohydrate improves cognitive measures postexercise, but not performance in trained females. *Med Sci Sports Exerc* 56: 725–736, 2024. doi:10.1249/MSS.0000000000003352.
21. **Clarke L**, **Russell SL**, **Spellanzon B**, **Maher JL**, **Betts JA**, **Gonzalez JT.** Ketone ester ingestion increases exogenous carbohydrate storage and lowers glycemia during post-exercise recovery: a randomised crossover trial. *Eur J Nutr* 64: 253, 2025. doi:10.1007/s00394-025-03784-w.
22. **McKay AKA**, **Stellingwerff T**, **Smith ES**, **Martin DT**, **Mujika I**, **Goosey-Tolfrey VL**, **Sheppard J**, **Burke LM.** Defining training and performance caliber: a participant classification framework. *Int J Sports Physiol Perform* 17: 317–331, 2022. doi:10.1123/ijspp.2021-0451.
23. **Fell JM**, **Hearris MA**, **Ellis DG**, **Moran JEP**, **Jevons EFP**, **Owens DJ**, **Strauss JA**, **Cocks M**, **Louis JB**, **Shepherd SO**, **Morton JP.** Carbohydrate improves exercise capacity but does not affect subcellular lipid droplet morphology, AMPK and p53 signalling in human skeletal muscle. *J Physiol* 599: 2823–2849, 2021 [Erratum in *J Physiol* 604: 660, 2026]. doi:10.1113/JP281127.
24. **Carte H**, **Jones AM**, **Doust JH.** Effect of 6 weeks of endurance training on the lactate minimum speed. *J Sports Sci* 17: 957–967, 1999. doi:10.1080/026404199365353.
25. **Kuipers H**, **Verstappen FTJ**, **Keizer HA**, **Geurten P**, **van Kranenburg G.** Variability of aerobic performance in the laboratory and its physiologic correlates. *Int J Sports Med* 6: 197–201, 1985. doi:10.1055/s-2008-1025839.
26. **Taylor C**, **Bartlett JD**, **van de Graaf CS**, **Louhelainen J**, **Coyne V**, **Iqbal Z**, **MacLaren DPM**, **Gregson W**, **Close GL**, **Morton JP.** Protein ingestion does not impair exercise-induced AMPK signalling when in a glycogen-depleted state: implications for train-low compete-high. *Eur J Appl Physiol* 113: 1457–1468, 2013. doi:10.1007/s00421-012-2574-7.
27. **Baldwin J**, **Snow RJ**, **Febbraio MA.** Effect of training status and relative exercise intensity on physiological responses in men. *Med Sci Sports Exerc* 32: 1648–1654, 2000. doi:10.1097/00005768-200009000-00020.
28. **Borg GA.** Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377–381, 1982.
29. **Wilson PB.** Frequency of chronic gastrointestinal distress in runners: validity and reliability of a retrospective questionnaire. *Int J Sport Nutr Exerc Metab* 27: 370–376, 2017. doi:10.1123/ijsnem.2016-0305.
30. **Pfeiffer B**, **Stellingwerff T**, **Hodgson AB**, **Randell R**, **PÖTtgen K**, **Res P**, **Jeukendrup AE.** Nutritional intake and gastrointestinal problems during competitive endurance events. *Med Sci Sports Exerc* 44: 344–351, 2012. doi:10.1249/MSS.0b013e31822dc809.
31. **Jeukendrup AE**, **Wallis GA.** Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med* 26 *Suppl* 1: S28–S37, 2005. doi:10.1055/s-2004-830512.
32. **Salek RM**, **Steinbeck C**, **Viant MR**, **Goodacre R**, **Dunn WB.** The role of reporting standards for metabolite annotation and identification in metabolomic studies. *Gigascience* 2: 13–13, 2013. doi:10.1186/2047-217X-2-13.
33. **Sumner LW**, **Amberg A**, **Barrett D**, **Beale MH**, **Beger R**, **Daykin CA**, **Fan TW-M**, **Fiehn O**, **Goodacre R**, **Griffin JL**, **Hankemeier T**, **Hardy N**, **Harnly J**, **Higashi R**, **Kopka J**, **Lane AN**, **Lindon JC**, **Marriott P**, **Nicholls AW**, **Reily MD**, **Thaden JJ**, **Viant MR.** Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* 3: 211–221, 2007. doi:10.1007/s11306-007-0082-2.
34. **Kohl SM**, **Klein MS**, **Hochrein J**, **Oefner PJ**, **Spang R**, **Gronwald W.** State-of-the art data normalization methods improve NMR-based metabolomic analysis. *Metabolomics* 8: 146–160, 2012. doi:10.1007/s11306-011-0350-z.
35. **Smolinska A**, **Blanchet L**, **Buydens LMC**, **Wijmenga SS.** NMR and pattern recognition methods in metabolomics: From data acquisition to biomarker discovery: a review. *Anal Chim Acta* 750: 82–97, 2012. doi:10.1016/j.aca.2012.05.049.
36. **Svart M**, **Rittig N**, **Pedersen SB**, **Jessen N**, **Møller N.** Oral 3-hydroxybutyrate ingestion decreases endogenous glucose production, lipolysis, and hormone-sensitive lipase phosphorylation in adipose tissue in men: a human randomized, controlled, crossover trial. *Diabet Med* 38: e14385, 2021. doi:10.1111/dme.14385.
37. **Taggart AKP**, **Kero J**, **Gan X**, **Cai T-Q**, **Cheng K**, **Ippolito M**, **Ren N**, **Kaplan R**, **Wu K**, **Wu T-J**, **Jin L**, **Liaw C**, **Chen R**, **Richman J**, **Connolly D**, **Offermanns S**, **Wright SD**, **Waters MG.** (D)-β-hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. *J Biol Chem* 280: 26649–26652, 2005. doi:10.1074/jbc.C500213200.
38. **Myette-Côté É**, **Neudorf H**, **Rafiei H**, **Clarke K**, **Little JP.** Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral glucose tolerance test in healthy young individuals. *J Physiol* 596: 1385–1395, 2018. doi:10.1113/JP275709.
39. **Mosquera-Lopez E**, **Louis J**, **Edwards JP**, **Pugh J**, **Viggars MR**, **Owens DJ**, **Areta JL.** Acute nutritional ketosis during early recovery from aerobic exercise does not affect skeletal muscle transcriptomic response in humans. *Eur J Appl Physiol* 126: 1021–1032, 2026. doi:10.1007/s00421-025-05987-9.
40. **Falkenhain K**, **Daraei A**, **Forbes SC**, **Little JP.** Effects of exogenous ketone supplementation on blood glucose: a systematic review and meta-analysis. *Adv Nutr* 13: 1697–1714, 2022. doi:10.1093/advances/nmac036.
41. **Mebane D**, **Madison LL.** Hypoglycemic action of ketones. i. effects of ketones on hepatic glucose output and peripheral glucose utilization. *J Lab Clin Med* 63: 177–192, 1964.
42. **Monteyne AJ**, **Falkenhain K**, **Whelehan G**, **Neudorf H**, **Abdelrahman DR**, **Murton AJ**, **Wall BT**, **Stephens FB**, **Little JP.** A ketone monoester drink reduces postprandial blood glucose concentrations in adults with type 2 diabetes: a randomised controlled trial. *Diabetologia* 67: 1107–1113, 2024. doi:10.1007/s00125-024-06122-7.
43. **Scott BE**, **Laursen PB**, **James LJ**, **Boxer B**, **Chandler Z**, **Lam E**, **Gascoyne T**, **Messenger J**, **Mears SA.** The effect of 1,3-butanediol and carbohydrate supplementation on running performance. *J Sci Med Sport* 22: 702–706, 2019. doi:10.1016/j.jsams.2018.11.027.
44. **Shaw DM**, **Merien F**, **Braakhuis A**, **Plews D**, **Laursen P**, **Dulson DK.** The effect of 1,3-butanediol on cycling time-trial performance. *Int J Sport Nutr Exerc Metab* 29: 466–473, 2019. doi:10.1123/ijsnem.2018-0284.
45. **(UCI) UCI.** UCI declaration concerning the use of ketone supplements by cyclists. https://www.uci.org/pressrelease/uci-declaration-concerning-the-use-of-ketone-supplements-by-cyclists/i4RNiOalWYxGqYtQ6h2rt [Oct 20].
46. **Sjölin J**, **Stjernström H**, **Henneberg S**, **Hambraeus L**, **Friman G.** Evaluation of urinary 3-methylhistidine excretion in infection by measurements of 1-methylhistidine and the creatinine ratios. *Am J Clin Nutr* 49: 62–70, 1989. doi:10.1093/ajcn/49.1.62.
47. **Long CL**, **Haverberg LN**, **Young VR**, **Kinney JM**, **Munro HN**, **Geiger JW.** Metabolism of 3-methylhistidine in man. *Metabolism* 24: 929–935, 1975. doi:10.1016/0026-0495(75)90084-0.
48. **Sheffield-Moore M**, **Dillon EL**, **Randolph KM**, **Casperson SL**, **White GR**, **Jennings K**, **Rathmacher J**, **Schuette S**, **Janghorbani M**, **Urban RJ**, **Hoang V**, **Willis M**, **Durham WJ.** Isotopic decay of urinary or plasma 3-methylhistidine as a potential biomarker of pathologic skeletal muscle loss. *J Cachexia Sarcopenia Muscle* 5: 19–25, 2014. doi:10.1007/s1339-013-0117-7.

49. **Churchward-Venne TA.** Anabolic and anticatabolic actions of ketone bodies on skeletal muscle: potential relevance in the management of skeletal muscle wasting. *Curr Opin Clin Nutr Metab Care* 28: 445–451, 2025. doi:10.1097/MCO.0000000000001164.
50. **García-Velázquez L**, **Massieu L.** The proteomic effects of ketone bodies: implications for proteostasis and brain proteinopathies. *Front Mol Neurosci* 16: 1214092, 2023. doi:10.3389/fnmol.2023.1214092.
51. **Ho A**, **Sinick J**, **Esko T**, **Fischer K**, **Menni C**, **Zierer J**, **Matey-Hernandez M**, **Fortney K**, **Morgen EK.** Circulating glucuronic acid predicts healthspan and longevity in humans and mice. *Aging (Albany NY)* 11: 7694–7706, 2019. doi:10.18632/aging.102281.
52. **Vi°na I**, **Linde R**, **Patetko A**, **Semjonovs P.** Glucuronic acid from fermented beverages: Biochemical functions in humans and its role in health protection. *Int J Res Rev Appl Sci* 14: 217–230, 2013.
53. **Dearlove DJ**, **Harrison OK**, **Hodson L**, **Jefferson A**, **Clarke K**, **Cox PJ.** The effect of blood ketone concentration and exercise intensity on exogenous ketone oxidation rates in athletes. *Med Sci Sports Exerc* 53: 505–516, 2021. doi:10.1249/MSS.0000000000002502.
54. **Frayn KN.** Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol Respir Environ Exerc Physiol* 55: 628–634, 1983. doi:10.1152/jappl.1983.55.2.628.
55. **Gonzalez JT**, **King AJ.** For flux sake: isotopic tracer methods of monitoring human carbohydrate metabolism during exercise. *Int J Sport Nutr Exerc Metab* 33: 60–70, 2023. doi:10.1123/ijsnem.2022-0170.
56. **Massicotte D**, **Peronnet F**, **Brisson G**, **Bakkouch K**, **Hillaire-Marcel C.** Oxidation of a glucose polymer during exercise: comparison with glucose and fructose. *J Appl Physiol (1985)* 66: 179–183, 1989. doi:10.1152/jappl.1989.66.1.179.
57. **Pugh JN**, **Wagenmakers AJM**, **Doran DA**, **Fleming SC**, **Fielding BA**, **Morton JP**, **Close GL.** Probiotic supplementation increases carbohydrate metabolism in trained male cyclists: a randomized, double-blind, placebo-controlled crossover trial. *Am J Physiol Endocrinol Metab* 318: E504–E513, 2020. doi:10.1152/ajpendo.00452.2019.
58. **Adopo E**, **Peronnet F**, **Massicotte D**, **Brisson GR**, **Hillaire-Marcel C.** Respective oxidation of exogenous glucose and fructose given in the same drink during exercise. *J Appl Physiol (1985)* 76: 1014–1019, 1994. doi:10.1152/jappl.1994.76.3.1014.
59. **Mougin L**, **Horner M**, **Edwards D**, **Nickels M**, **Taylor L**, **James LJ**, **Mears SA.** Heat stress impairs exogenous carbohydrate oxidation during prolonged running when maintaining euhydration. *J Appl Physiol (1985)* 139: 1436–1446, 2025. doi:10.1152/japplphysiol.00873.2025.
60. **Podlogar T**, **Bokal Š**, **Cirnski S**, **Wallis GA.** Increased exogenous but unaltered endogenous carbohydrate oxidation with combined fructose-maltodextrin ingested at 120 g h−1 versus 90 g h−1 at different ratios. *Eur J Appl Physiol* 122: 2393–2401, 2022. doi:10.1007/s00421-022-05019-w.
61. **Jentjens RLPG**, **Jeukendrup AE.** High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. *Br J Nutr* 93: 485–492, 2005. doi:10.1079/bjn20041368.
62. **Jentjens RLPG**, **Underwood K**, **Achten J**, **Currell K**, **Mann CH**, **Jeukendrup AE.** Exogenous carbohydrate oxidation rates are elevated after combined ingestion of glucose and fructose during exercise in the heat. *J Appl Physiol (1985)* 100: 807–816, 2006. doi:10.1152/japplphysiol.00322.2005.
63. **McCarthy DG**, **Bostad W**, **Bone J**, **Powley FJ**, **Richards DL**, **Gibala MJ.** Effect of acute ketone monoester ingestion on cardiorespiratory responses to exercise and the influence of blood acidosis. *Med Sci Sports Exerc* 55: 1286–1295, 2023. doi:10.1249/MSS.0000000000003141.
64. **Bone J**, **Baumgarten S**, **McCarthy DG**, **Bostad W**, **Richards DL**, **Gibala MJ.** Acute ketone monoester supplementation does not change exercise efficiency during incremental cycling in trained individuals. *Med Sci Sports Exerc* 57: 163–172, 2025. doi:10.1249/MSS.0000000000003532.