american physiological society

JOURNAL OF APPLIED PHYSIOLOGY

J Appl Physiol 138: 1309–1320, 2025.
First published May 2, 2025; doi:10.1152/japplphysiol.00097.2025

RESEARCH ARTICLE

Ketone ester ingestion impairs exercise performance without impacting cognitive function or circulating EPO during acute hypoxic exposure

Myrthe Stalmans,[1] Domen Tominec,[2] Wout Lauriks,[1] Ruben Robberechts,[1,3] Monique Ramaekers,[1] Tadej Debevec,[2,4] and Chiel Poffé[1,3]

[1]*Exercise Physiology Research Group, Department of Movement Sciences, KU Leuven, Leuven, Belgium;* [2]*Faculty of Sport, University of Ljubljana, Ljubljana, Slovenia;* [3]*REVAL – Rehabilitation Research Center, Faculty of Rehabilitation Sciences, Hasselt University, Diepenbeek, Belgium; and* [4]*Department of Automatics, Biocybernetics and Robotics, Jožef Stefan Institute, Ljubljana, Slovenia*

Abstract

Altitude-induced hypoxemia impairs exercise performance and cognition. Interestingly, ketone ester (KE) ingestion may attenuate hypoxemia, which likely explains the observation that KE impairs high-intensity exercise performance in normoxia but not in hypoxia. Moreover, KE was reported to attenuate cognitive decline at extreme altitudes (~6,100 m). Given that hypoxemia is unaffected by KE in milder conditions, the impact of KE on cognition and performance in the absence of elevated oxygenation remains unknown. As KE may increase postexercise circulating [erythropoietin] ([EPO]) at sea level, we also assessed if KE might augment the blood [EPO] response after hypoxic exercise. In a double-blind, cross-over design, 13 healthy, male participants completed two 5.5-h sessions at 4,000-m simulated altitude while receiving either KE or placebo (CON). Throughout a graded exercise test (EX_{MAX}) after 1.5 h, and a submaximal exercise bout (EX_{SUBMAX}) after 3 h, blood and tissue oxygenation, ventilatory parameters, and acid-base balance were evaluated. Other measurements included cognitive function and blood [EPO]. KE reduced power output achieved during EX_{MAX} by 3.6%, whereas blood and cerebral oxygenation were similar. KE ingestion lowered blood pH, [HCO_3^-], pCO_2, and [glucose], but did not impact cognitive function. In both KE and CON, circulating [EPO] increased by ~56% after 5 h. These results indicate that KE ingestion impairs high-intensity exercise performance, at least if not compensated by elevated oxygenation. A progressively increasing oxygenation upon KE was unable to protect against hypoxia-induced cognitive declines and potentially counteracted a KE-induced augmentation of circulating [EPO].

NEW & NOTEWORTHY This study is the first to show that KE ingestion impairs exercise performance in hypoxia, at least when KE does not alleviate hypoxemia. Despite a subsequent, progressive increase in oxygenation upon KE after 3–4 h, this does not protect against hypoxia-induced cognitive declines. Although studies in normoxia show potential of KE to increase blood [erythropoietin], we identified that KE ingestion fails to augment the increase in blood [erythropoietin] through hypoxic exposure and exercise.

cognition; erythropoietin (EPO); exercise performance; hypoxia; ketones

INTRODUCTION

Acute exposure to high altitude results in insufficient availability of oxygen to keep up with the oxygen demand of the human body, thereby causing the development of hypoxemia. This negatively affects multiple functional outcomes including exercise performance and cognitive function. For instance, earlier studies indicated that high-intensity exercise performance decreased by 14% per 1,000-m elevation (1), whereas acute hypoxia has also been shown to impair reaction time (2), visual information processing (3), and working memory (4). In multiple activities at high altitudes, such as mountaineering, skiing, or military operations, it is however of primary importance to achieve optimal physical performance while simultaneously executing critical cognitive tasks. Therefore, there is keen interest in identifying strategies that mitigate the negative impact of (acute) hypoxia on both physical performance and cognitive function. The primary factor underlying the negative effects of acute hypoxia is a reduction in blood oxygen saturation (5, 6). As such, alleviating the negative impact of acute hypoxia can most likely be achieved by strategies that mitigate hypoxia-induced oxygen desaturation.

Interestingly, previous work from our and other laboratories suggests that increasing blood ketone bodies via ketone monoester (KE) ingestion is effective in attenuating hypoxia-induced declines in blood oxygen saturation both at rest (7, 8) and during exercise (9, 10), thereby potentially mitigating acute mountain sickness (AMS) development at a simulated altitude of ~4,000 m (7). The ability of KE to attenuate hypoxemia primarily results from the accompanying

Correspondence: M. Stalmans (myrthe.stalmans@kuleuven.be); C. Poffé (chiel.poffe@uhasselt.be).
Submitted 4 February 2025 / Revised 17 March 2025 / Accepted 24 April 2025

metabolic acidosis and concomitant increase in ventilatory drive (7, 9). Although the underlying mechanisms remain unknown, KE ingestion has been shown to only attenuate hypoxemia under some conditions, with contrasting findings contesting the hypotheses that this may depend on the duration of hypoxic exposure [~3–4 h (7, 11) vs. 15 min (8, 10)], or the degree of blood desaturation [below (7, 8, 10, 11) or above (9) ~85%].

Initial research suggested that exogenous ketosis could induce ergogenic effects at sea level (12); however, this narrative shifted as more ecologically valid studies often failed to replicate these results. Despite several contrasting findings, current evidence indicates that high-intensity endurance performance at sea is not improved (13–17) and potentially even impaired by ingestion of ketone monoesters (18, 19), diesters (20), or salts (21), if not compensated by coingestion of bicarbonate (22, 23). This is primarily attributed to a ketosis-induced reduction in extracellular buffering capacity (18, 23) and glycolytic ATP production (12). To our knowledge, only one earlier report from our laboratory investigated the effect of exogenous ketosis on high-intensity endurance performance in hypoxic conditions, where KE ingestion did not impair 15-min time-trial performance at a simulated altitude of 3,000 m (9). Although we acknowledge substantial methodological differences with previous (normoxic) reports, such as time trial duration, pre-test exercise load, and supplementation protocol, these divergent effects may result from the KE-induced attenuated hypoxemia, which potentially compensated for the "intrinsic" ergolytic effect of KE on high-intensity exercise performance. However, the impact of KE on performance upon acute hypoxic exposure at a time when the ketone-induced attenuation of hypoxemia has not (yet) been established, remains to be determined.

Acute hypoxia impairs cognitive functioning, including reaction time (2), visual information processing (3), and (spatial) working memory (2, 4, 24, 25). KE may upregulate normal cognitive function in various conditions (26–28) but has also been shown to attenuate extreme endurance (100-km ultrarun) exercise-induced declines in reaction or movement time, which was supposedly related to increased circulating dopamine concentrations (29). More recently, it was observed that KE also attenuated the reduction in cognitive efficiency that was induced by 20-min exposure to a reduced O_2 breathing device ($F_{I_{O_2}}$: 9.7%, ~6,100 m) (8).

Apart from the acute, negative effects on performance and cognition, one of the primary adaptive responses to hypoxia is an increase in circulating erythropoietin (EPO), which ultimately expands red blood cell mass to, in the long term, alleviate hypoxemia (30). Recent studies indicated that both acute (31) and longer-term (32) KE ingestion may increase circulating [EPO] by 20%–25% in normoxic conditions. For instance, KE ingestion gradually increased serum EPO concentrations starting 2 h after a 60-min high intensity interval training (31). Nevertheless, it remains to be determined if KE may further augment the erythropoietic response to both hypoxic exposure and hypoxic exercise, thereby augmenting beneficial adaptations to hypoxia.

Against this background, we aimed to evaluate the impact of KE on exercise performance and cognitive function and to identify if KE may further increase EPO, under hypoxic conditions containing intermittent low- and high-intensity exercise. We hypothesize that in the absence of elevated oxygenation, KE ingestion will further impair exercise performance upon acute exposure to hypoxia. Moreover, we hypothesize that KE may improve cognitive function or attenuate gradual hypoxia- or exercise-induced cognitive declines. Given the recent evidence in normoxic conditions, we hypothesize that ingestion of KE will further augment circulating EPO concentrations.

METHODS

Study Design and Participants

Fourteen healthy male participants were recruited to partake in a randomized, double-blind, placebo-controlled, crossover study. This research was executed in the context of a larger project of which the details have been reported elsewhere (7). The study was approved by the Ethics Committee Research UZ/KU Leuven (B3222022000810), preregistered at www.clinicaltrials.gov (NCT05588427), and conducted in accordance with the Declaration of Helsinki guidelines. Potential candidates were required to conform to the following inclusion criteria: *1*) age: 18–35 yr, *2*) body mass index (BMI): 18–25 $kg \cdot m^{-2}$, and *3*) regularly physically active: 2–7 $h \cdot wk^{-1}$. Smokers, candidates with a history of altitude-sensitive pathologies, or those with exposure to altitudes above 1,500 m in the 3 mo preceding the study were excluded. Although we originally planned to include female participants, the limited availability of the hypoxic testing facility did not allow appropriate, menstrual phase-based scheduling of the sessions to standardize hormonal balance across the cycle. All participants provided written informed consent and were medically screened before inclusion in the study. One participant developed severe AMS after only 1 h in hypoxia in their CON session and was therefore excluded from data analysis, resulting in a sample size of $n = 13$.

The larger study involved a 29-h protocol during which participants resided at a simulated altitude of 4,000 m ($F_{I_{O_2}}$: 12.7%) during the first 26 h, followed by a prompt transition to 4,500 m ($F_{I_{O_2}}$: 11.8%) for the final 3 h of the protocol. Throughout the protocol, participants received either KE drinks to induce constant ketosis during the diurnal parts of the protocol, or a placebo (CON). Following a 1-wk washout period, participants performed an identical session with the other drink. During both sessions, participants regularly performed submaximal and maximal exercise bouts on a cycling ergometer, with the submaximal exercise bouts being designed to simulate the workload associated with normal ascend rates (33). The present study (Fig. 1) focuses on the first 5.5 h of the protocol wherein participants performed a maximal (at 1.5 h) and submaximal (at 3 h) exercise bout, and three times a cognitive test battery (at 1, 2, and 5 h).

Preliminary Testing

Before the first experimental session, participants performed an incremental cycling test in normoxic conditions. This consisted of a 10-min warm-up (WU) at 70 W, followed by a ramp protocol of 100 W + 10 W/30 s until voluntary exhaustion. Maximal oxygen consumption rate ($\dot{V}O_{2max}$) was determined using indirect calorimetry (Cortex Metalyzer

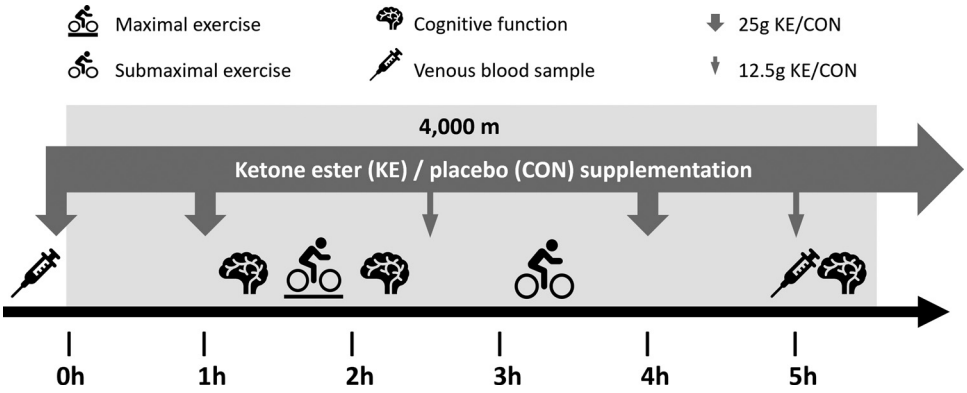

Figure 1. Outline of the experimental protocol. In a double-blind, randomized, crossover design, 13 participants took part in a 5.5-h normobaric hypoxic protocol at 4,000 m. Throughout the protocol, participants regularly received either ketone ester (KE) or placebo (CON) supplements. In each session, a maximal (EX_{MAX}, 10 min warm-up + ramp protocol) and submaximal (EX_{SUBMAX}, 30 min) exercise bout were completed, and the physiological response was characterized by measurements of blood oxygen saturation, cerebral and muscular oxygenation status, and respiratory gas exchange. Capillary blood samples were collected before and during EX_{MAX} and EX_{SUBMAX} for determination of acid-base balance and capillary blood gases. Venous blood samples were collected before hypoxic entry and after 5 h in hypoxia. Moreover, immediately before and after EX_{MAX}, as well as after 5 h in hypoxia, cognitive function was assessed.

IIIb, Leipzig, Germany) and was defined as the highest $\dot{V}O_2$ value over a 15-s period. After 15 min of passive recovery, participants were familiarized with the cognitive tests (see *Cognitive Function Assessment*).

Nutritional Intervention

Throughout the KE session, participants ingested the ketone monoester (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (KetoneAid Inc., Falls Church, Virginia; ~4.7 kcal·g^{-1}) in boluses of either 25 g (30 min before entering hypoxia, as well as after 1, and 4 h in hypoxia) or 12.5 g (after 2.5 and 5 h in hypoxia). In CON, participants consumed identical boluses of a nonisocaloric, taste and viscosity-matched placebo consisting of 12.5% wt/vol collagen (6d Sports Nutrition, Oudenaarde, Belgium) and 1 mM bitter sucrose octaacetate (Sigma-Aldrich, Bornem, Belgium) dissolved in water. Furthermore, 5% wt/vol sucralose (MyProtein, New York City) and 1.0% vol/vol strawberry flavor drops (MyProtein, New York City) were added to both supplements to soften the bitter taste. Supplements were provided in nontransparent 50 mL tubes to avoid potential visual identification. Dietary intake was standardized from the evening before until the end of the experimental protocol and generally included carbohydrate-rich foods [~60% carbohydrate, ~20% fat, ~20% protein, full details have been reported earlier (7)].

Exercise Bouts and Assessment of Perceived Exertion

The maximal exercise bout (EX_{MAX}) was performed after 1.5 h at altitude and consisted of a 10-min warm-up (WU) at 1.5 W·kg^{-1} followed by a ramp protocol of 100 W + 10 W/30 s until voluntary exhaustion. After 3 h at altitude, participants performed a 30-min submaximal exercise bout (EX_{SUBMAX}), which consisted of constant cycling at 1.5 W·kg^{-1}. Upon completion of both exercise bouts, the rating of perceived exertion (RPE) was assessed using a 6–20 Borg scale. Experimental measurements were performed both during maximal and submaximal exercise [see *Blood and Tissue (Prefrontal Cortex and Skeletal Muscle) Oxygenation Status* and *Ventilatory Measurements*].

Cognitive Function Assessment

Cognitive function was assessed *1*) after 1 h in hypoxia (i.e., before EX_{MAX} at rest), *2*) after 2 h in hypoxia (i.e., after EX_{MAX}), as well as *3*) after 5 h in hypoxia (i.e., at rest). Therefore, we used a cognitive test battery (Cantab, Cambridge Cognition, Cambridge, UK) consisting of three validated tests to evaluate *1*) reaction time (RTI), *2*) rapid visual information processing (RVP), and *3*) spatial working memory (SWM). These tests were specifically selected to evaluate relevant alterations caused by either hypoxia or ketone ester supplementation (29, 34). All tests were conducted on a tablet (Lenovo Table M10 FHD plus, Lenovo, Hongkong, China) that was placed on an inclined screen holder, whereas participants were seated on a comfortable chair, facing a wall, and in the absence of other individuals. Participants were instructed to perform all tests with the index finger of the dominant hand, and to contact the researchers upon completion of all three tests. A detailed explanation of the task procedures is available at www.cambridgecognition.com/cantab/cognitive-tests/. Outcome parameters of each test were *1*) RTI: median reaction time (RTI-MRT), median movement time (RTI-MMT), and number of errors out of 30 (RTI-E); *2*) RVP: mean response latency (RVP-MRL), number of correct responses (hits) out of 54 (RVP-H), and false alarms out of 54 (RVP-FA), and *3*) SWM: strategy score (SWM-SS) and number of errors (SWM-E).

Blood and Tissue (Prefrontal Cortex and Skeletal Muscle) Oxygenation Status

Blood (Sp_{O_2}), cerebral, and skeletal muscle oxygenation status were measured *1*) at the start of EX_{MAX} (START, i.e., before WU), as well as *2*) during the final min of WU (END WU), and *3*) during the final minute before exhaustion (PEAK), and *4*) at 10-min intervals during EX_{SUBMAX}.

Sp_{O_2} measurements were performed at 2 Hz using a pulse oximeter (Nellcor PM10N, Medtronic, Minneapolis) with an infrared sensor placed ~2 cm above the left eyebrow. Cerebral and skeletal muscle oxygenation status were assessed using near-infrared spectroscopy (NIRS), by analysis of tissue oxygenation index (cTOI and mTOI, resp.). The

probes of a NIRO-200 spectrometer (Hamamatsu, Japan) were attached ~2 cm above the right eyebrow (cerebral oxygenation) and centrally on the belly of the right m. vastus lateralis (skeletal muscle oxygenation). Interoptode distance was fixed at 4 cm to ensure a constant penetration depth of ~2 cm into the muscle/brain tissue, guaranteed by inserting the emitter and detector probes in a dark-colored rubber spacer. These spacers were attached using an elastic nontransparent bandage and double-sided adhesive tape to prevent displacement or interference from external light. At the start of each experimental session, the involved skin was shaved and cleaned to exclude any signal disturbance by hair or impurities, and the contour lines of the rubber spacer were marked on the skin before the first session to ensure identical positioning for every measurement. Participants were asked to preserve and refresh these marks during the washout period to maintain identical positioning during the second session. After the experimental procedures, NIRS data were processed (MATLAB R2023a, The MathWorks, Natick, MA) using a fourth-order Butterworth filter with a cut-off frequency of 0.05 Hz (9) and analyzed over 1-min long chunks during the maximal ramp protocol or over 10-min long time chunks during the submaximal exercise protocol.

Ventilatory Measurements

Indirect calorimetry (Cortex Metalyzer IIIb, Leipzig, Germany) was used throughout EX_{MAX} and during the final 10 min of EX_{SUBMAX}. Gas exchange data [i.e., minute ventilation ($\dot{V}_E$), oxygen uptake rate ($\dot{V}O_2$), and carbon dioxide production rate ($\dot{V}CO_2$)], as well as breathing frequency and tidal volume, were collected breath-by-breath. Data are presented as mean values of the final 5 min of WU (END WU), and the mean values of the final 30 s before exhaustion for EX_{MAX} (PEAK), and as mean values of the final 5 min during EX_{SUBMAX} (END).

Capillary Blood Samples and Analyses

Immediately before the first supplement and 30 min after each supplement, capillary blood samples were obtained from a hyperemic earlobe for immediate determination of D-β-hydroxybutyrate (βHB, GlucoMen Areo 2K-meter with β-ketone sensor strips, A. Menarini Diagnostics, Firenze, Italy). To guarantee successful blinding, these measurements were performed by a researcher who was otherwise not involved in the study. In addition, 70-μL capillary blood was collected from a hyperemic earlobe into a capillary tube (safeCLINITUBE, Radiometer Medical ApS, Copenhagen, Denmark) during both EX_{MAX} and EX_{SUBMAX}. After immediate mixing for 10 s, samples were analyzed for acid-base balance, pO_2, pCO_2, and p50 (ABL90 FLEX analyzer, Radiometer Medical ApS, Copenhagen, Denmark). Timepoints during EX_{MAX} were *1*) at start (START), *2*) during the final minute of WU (END WU), and *3*) immediately, and *4*) 2 min after volitional exhaustion (PEAK). For EX_{SUBMAX}, measurements were performed both *1*) at the start (START), and *2*) during the final minute of EX_{SUBMAX} (END). For EX_{MAX}, the capillary sample obtained immediately after exhaustion was used as a PEAK value in further analyses for all parameters, except for blood [lactate] and blood pH. For these parameters, the sample with respectively the highest and lowest values were selected between the sample immediately upon exhaustion, and the sample 2 min after exhaustion.

Venous Blood Samples

Venous blood samples were obtained from an antecubital vein (Venoject, Terumo, Tokyo, Japan) in a fasted state before hypoxic entry (baseline), as well as after 5 h in the hypoxic protocol. The samples were collected into vacuum tubes containing Silica Clot Activator [Becton Dickinson (BD) Vacutainer, Eysins, Switzerland]. Tubes were centrifuged (1,500 rpm for 10 min at 4°C) and the obtained supernatant was stored at −80°C until later analysis. Subsequently, a commercially available enzyme-linked immunosorbent assay (ELISA) was performed to determine serum EPO concentrations (%CV = 5.3%; DY286-05, R&D Systems, Minneapolis, MN).

Statistical Analyses and Sample Size Calculation

All statistical analyses were performed in GraphPad Prism version 10.3.1 (GraphPad Software, La Jolla, CA). For data measured at a single timepoint per experimental session (e.g., peak power achieved during EX_{MAX} and ventilatory parameters during EX_{SUBMAX}), normality was evaluated using a D'Agostino and Pearson test. Normally distributed data were evaluated using a paired *t* test, whereas a nonparametric Wilcoxon matched-pairs signed rank test was used for nonnormal distributed data. Measurements that were performed at multiple timepoints per experimental session were analyzed using a two-way repeated-measures analysis of variance (ANOVA) or using a mixed-effects model in case of missing data, evaluating differences between KE and CON over time. In case of a significant interaction effect, post hoc analyses were performed using Šidák's correction. For significant analyses, effect sizes were calculated as Hedge's *g* for *t* tests, as η_p^2 for ANOVA analyses, and as r^2 for mixed-effects analyses. Reported *P* values generally refer to these post hoc analyses and otherwise, *P* values for main effects were included. All data are presented as means ± SD unless otherwise stated. Statistical significance was defined as $P < 0.05$. An a-priori sample size calculation was based on an earlier study from our laboratory, which reported increased blood saturation upon KE during exercise in hypoxia (9), indicating a required sample size of $n = 10$ per condition (effect size dz: 0.52; α error: 0.05; power: 0.80; two-sided; ANOVA repeated-measures, within-between interaction; elaborately described in Ref. (7). Conversely, the current article hypothesizes that ingestion of KE impairs exercise performance at a timepoint when oxygenation is not improved by KE. In this regard, a power calculation based on the previously reported KE-induced drop in mean power output during a 30-min time trial (18) recommended a sample size of $n = 8$ (effect size dz: 2.53, α error: 0.05, power: 0.8, paired two-sided *t* test). Given the higher sample size required, as well as the primordial role of oxygen desaturation in exercise performance, cognitive function, but also the production of EPO, this calculation was deemed appropriate for the current article, which included a sample size of $n = 13$.

RESULTS

Ketone Ester Intake Increased Blood Ketone Concentrations

As previously reported (7), blood [βHB] was low (~0.4 mM) before hypoxic entry in both conditions. KE ingestion induced a stable ketosis during the diurnal part of the protocol resulting in consistent blood [βHB] of ~2 to 4 mM. More specifically, blood [βHB] in the KE condition were 2.9 ± 0.5 mM (upon hypoxic entry, 0 h), 3.0 ± 1.0 mM (start of EX_{MAX}, 1.5 h), 3.2 ± 0.9 mM (start of EX_{SUBMAX}, 3 h), and 4.5 ± 1.1 mM (30 min before the final cognitive test, 4.5 h). Conversely, blood [βHB] remained below 0.5 mM at all these timepoints in CON ($P < 0.001$ for time × KE effect, $\eta_p^2 = 0.84$).

KE Impaired Maximal Exercise Performance without Impacting Cognitive Function or Perceived Exertion during Either Submaximal or Maximal Exercise

Peak power output reached during EX_{MAX} was 3.6% or 10 W lower in KE compared with CON ($P = 0.041$, $g = -0.33$, 95% CI: −0.5 to −19.3 W, Fig. 2, *A* and *B*). Similarly, the average duration of EX_{MAX} decreased by 38 s upon KE ($P = 0.014$, $g = -0.44$, 95% CI: −9 to −67 s, Fig. 2*C*). Conversely, RPE scores upon completion of both EX_{MAX} (KE: 18 ± 3 vs. CON: 19 ± 2, $P = 0.813$) and EX_{SUBMAX} (KE: 12 ± 2 vs. CON: 12 ± 3, $P > 0.999$) were similar between KE and CON. A main time effect was observed for median reaction time ($P = 0.037$, $\eta_p^2 = 0.21$) and median movement time ($P = 0.018$, $\eta_p^2 = 0.35$) during RTI (Fig. 2, *D* and *E*, resp.). In both experimental conditions, values were similar before and after EX_{MAX} ($P = 0.623$ and $P = 0.147$ for 2 h vs. 1 h, resp.), but increased by, respectively, 3% and 9% after 5 h in hypoxia ($P = 0.032$ and $P = 0.015$ for 5 h vs. 1 h, resp.). For total errors during RTI, neither a main nor interaction effect was observed, indicating that the number of errors was low (~ 85% of tests with either 0 or 1 error, overall range: 0–5) in both conditions at all time points. For the RVP task, the number of false alarms increased in response to the maximal exercise bout [1 h: 1 (1–2) median (IQR) vs. 2 h: 3 (2–3), $P = 0.005$ for 2 h vs. 1 h], and reverted back to pre-exercise values at 5 h [2 (1–2)] in both conditions ($P = 0.419$ for 5 h vs. 1 h; $P = 0.006$ for main time effect, $\eta_p^2 = 0.34$, Fig. 2*F*). Conversely, the number of hits (~45) and response latency (~490 ms) were unaffected by time in hypoxia ($P > 0.155$ for main time effects) or KE ($P > 0.272$ for main KE and time × KE effects). Also, for the SWM task, the number of errors and strategy were not affected by time in hypoxia ($P > 0.133$ for main time effect) or KE ($P > 0.576$ for main KE or time × KE effects).

KE Attenuated Muscular Deoxygenation during Maximal and Submaximal Exercise, and Alleviated Blood and Cerebral Deoxygenation during Submaximal Exercise

Maximal exercise bout.

In both conditions, Sp_{O_2} (Fig. 3*A*) dropped throughout WU from ~83% to ~73% ($P < 0.001$ for END WU vs. START), thereafter slightly further dropping to ~70% upon PEAK ($P = 0.020$ for PEAK vs. END WU; $P < 0.001$ for main time

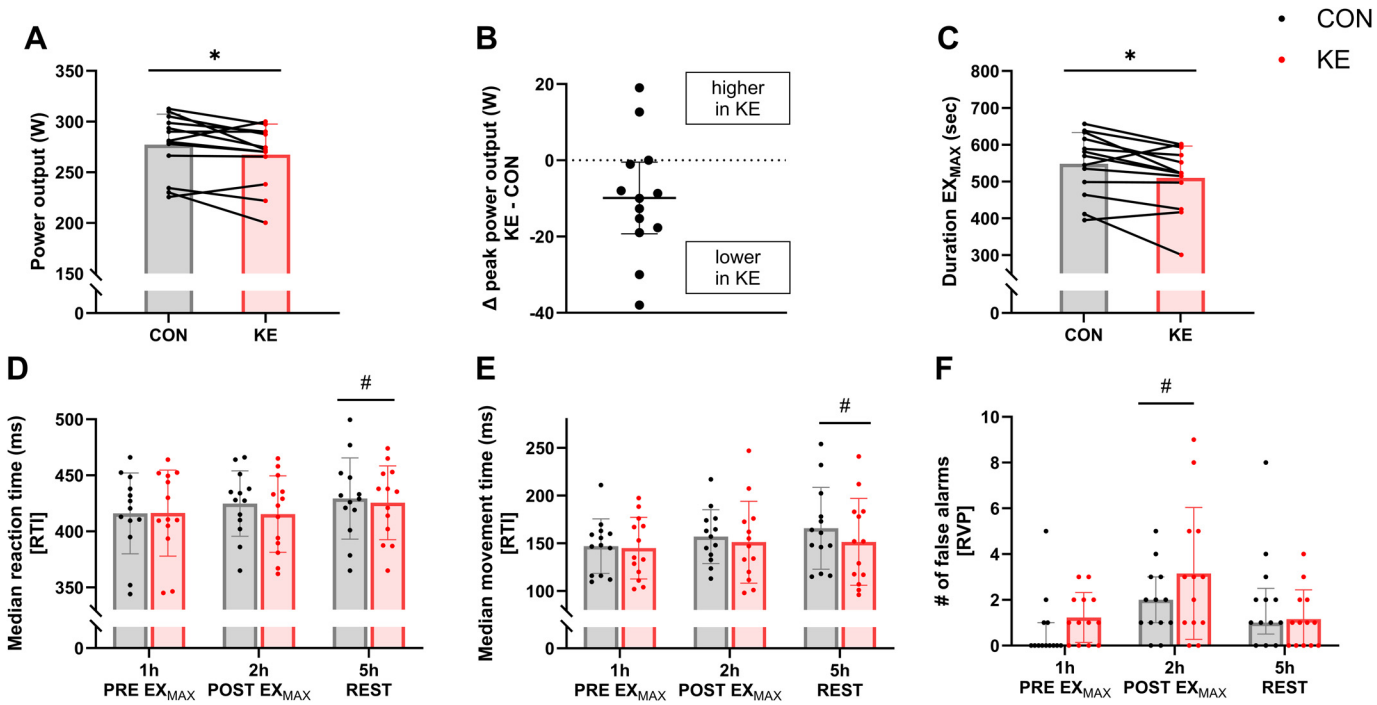

Figure 2. Effect of ketone ester (KE) ingestion on exercise performance and cognitive function. Data are represented as means ± SD along with individual values ($n = 13$) for peak power output achieved during a graded exercise test (EX_{MAX}) (*A*), and individual differences between participants' KE and CON sessions with means ± 95% CI (*B*). Means ± SD along with individual values are also shown for the duration of EX_{MAX} (*C*), but also for median reaction time (*D*) and median movement time (*E*), both during a reaction time task (RTI), as well as the number of false alarms out of 54 during a visual information processing task (RVP) (*F*). During 5.5 h at 4,000 m altitude (normobaric hypoxia) involving intermittent KE (red) or placebo (CON, black) ingestion, participants completed a maximal graded exercise test after 1.5 h in hypoxia. Before (PRE EX_{MAX}) and after (POST EX_{MAX}) this maximal test, as well as after 5 h in hypoxia (REST), a cognitive test battery was performed. #$P < 0.05$ vs. baseline (PRE EX_{MAX}) in both conditions; *$P < 0.05$ for KE vs. CON.

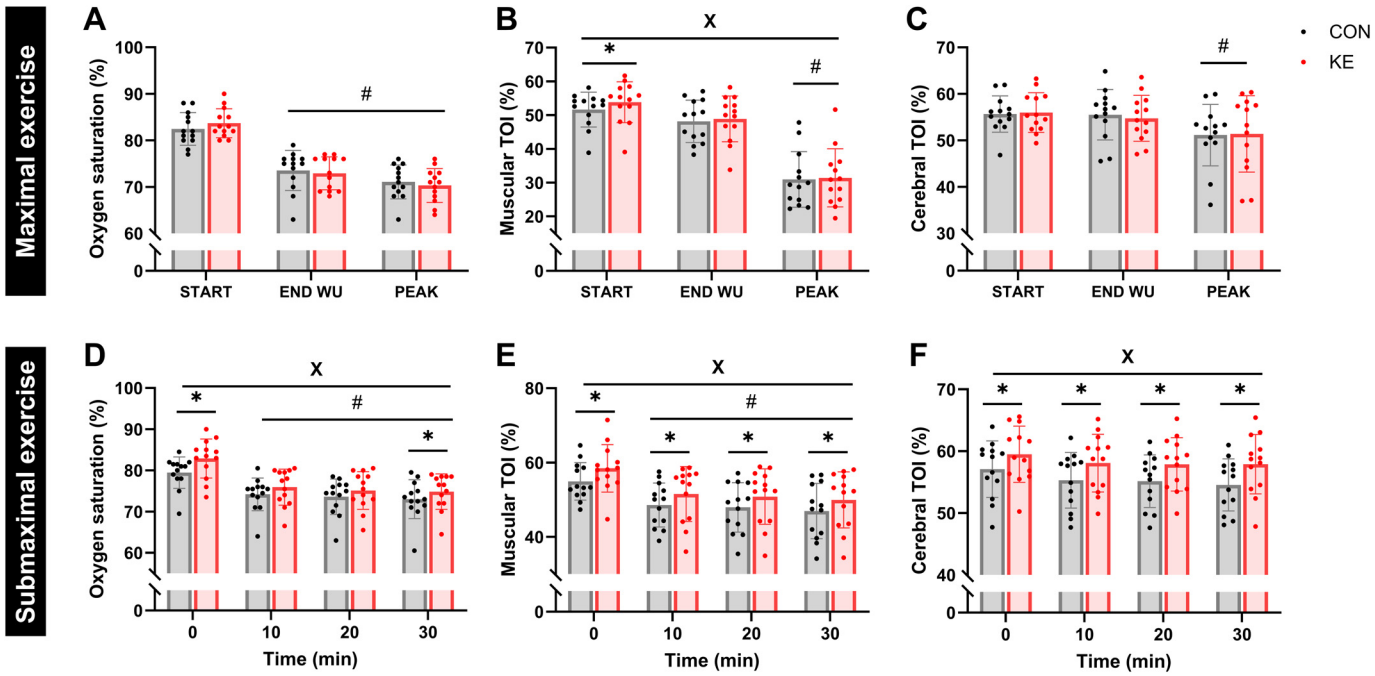

Figure 3. Effect of ketone ester (KE) ingestion on blood and tissue oxygenation during exercise. Data are represented as means ± SD along with individual values ($n = 13$) for blood oxygen saturation (*A*), muscular tissue oxygenation index (TOI) (*B*), and cerebral TOI (*C*), all during maximal exercise, as well as for blood oxygen saturation (*D*), muscular TOI (*E*), and cerebral TOI during submaximal exercise (*F*). During a 5.5-h normobaric hypoxic protocol involving intermittent KE (red) or placebo (CON, black) ingestion, participants completed a graded maximal exercise test after 1.5 h and a submaximal exercise bout after 3 h in hypoxia. Data are shown before the start (START), during the final minute of warm-up (END WU), and during the final minute before exhaustion (PEAK) of the maximal exercise, as well as at 10-min intervals during the submaximal exercise bout. #$P < 0.05$ vs. baseline (i.e., START or 0 min) for both conditions; X$P < 0.05$ for main KE effect; *$P < 0.05$ KE vs. CON for indicated timepoints.

effect, $\eta_p^2 = 0.95$). Both mTOI (Fig. 3*B*) and cTOI (Fig. 3*C*) were stable during WU ($P = 0.062$ and $P = 0.831$ for END WU vs. START, resp.) but subsequently dropped to a similar extent in both conditions upon PEAK ($P < 0.001$ and $P = 0.003$ for PEAK vs. START; $P < 0.001$ and $P = 0.002$ for main time effect, $\eta_p^2 = 0.99$ and $\eta_p^2 = 0.84$, resp.). Yet, a main KE effect for mTOI ($P = 0.049$, $\eta_p^2 = 0.21$) was likely due to higher mTOI values at START in KE (53.8 ± 6.1%) versus CON (51.6 ± 5.1%) (START: $P = 0.029$, END WU: $P = 0.747$, PEAK: $P = 0.913$ for KE vs. CON).

Submaximal exercise bout.

In both conditions, Sp_{O_2} (Fig. 3*D*), mTOI (Fig. 3*E*), and cTOI (Fig. 3*F*) decreased by 10%, 5%, and 3%, respectively, during EX_{SUBMAX} ($P < 0.001$ for main time effects; $\eta_p^2 = 0.89$, $\eta_p^2 = 0.94$, and $\eta_p^2 = 0.71$, resp.). However, a main KE effect was observed for Sp_{O_2}, mTOI, and cTOI throughout EX_{SUBMAX} ($P = 0.015$, $P = 0.008$, and $P = 0.016$; $\eta_p^2 = 0.51$, $\eta_p^2 = 0.77$, and $\eta_p^2 = 0.88$, resp.), as ingestion of KE increased Sp_{O_2} (START: $P < 0.001$, 10 min: $P = 0.069$, 20 min: $P = 0.148$, 30 min: $P = 0.047$ for KE vs. CON), and increased mTOI and cTOI ($P < 0.001$ for KE vs. CON for all).

KE Increased Blood p50 and Lowered Blood pCO_2, [Glucose], pH, [HCO_3^-], and [Lactate] during Submaximal and Maximal Exercise

Maximal exercise bout.

Although pCO_2 (Fig. 4*A*) decreased in both conditions by ~2 mmHg throughout WU ($P < 0.001$ for END WU vs. START) and further dropped upon PEAK ($P < 0.001$ for PEAK vs. END WU; $P < 0.001$ for main time effect, $r^2 = 0.87$), ingestion of KE lowered pCO_2 with ~2 mmHg ($P = 0.009$ for main KE effect, $r^2 = 0.45$, START: $P = 0.013$, END WU: $P = 0.091$, PEAK: $P = 0.929$ for KE vs. CON). pO_2 was higher upon KE ingestion before the onset of EX_{MAX} (CON: 42.0 ± 2.7 mmHg, KE: 44.6 ± 2.8 mmHg, $P = 0.003$ for KE vs. CON); however, this difference disappeared after WU (CON: 36.3 ± 2.9 mmHg, KE: 36.1 ± 2.6 mmHg, $P = 0.999$ for KE vs. CON) and upon PEAK (CON: 47.1 ± 4.0 mmHg, KE: 46.3 ± 2.7 mmHg, $P = 0.939$ for KE vs. CON; $P = 0.027$ for time × KE effect, $r^2 = 0.34$). p50 was higher throughout EX_{MAX} upon KE ingestion ($P = 0.010$ for main KE effect, $\eta_p^2 = 0.38$, START: $P = 0.020$, END WU: $P = 0.099$, PEAK: $P = 0.464$ for KE vs. CON). In both conditions, p50 remained stable during WU (CON: 25.4 ± 1.1 mmHg vs. KE: 26.3 ± 0.8 mmHg at START, $P = 0.947$ for END WU vs. START), but increased by 20% upon PEAK (CON: 31.0 ± 1.7 mmHg vs. KE: 31.5 ± 1.9 mmHg, $P < 0.001$ for PEAK vs. END WU; $P < 0.001$ for main time effect, $\eta_p^2 = 0.96$). The difference between pO_2 and p50 decreased during WU in both conditions (CON: 16.6 ± 3.0 at START vs. 11.0 ± 2.8 at END WU; KE: 18.3 ± 3.4 at START vs. 9.8 ± 2.5 at END WU; $P < 0.001$ for END WU vs. START for both), and remained lower at PEAK in KE (14.9 ± 14.9, $P = 0.011$ for PEAK vs. START), whereas returning to baseline values in CON (16.1 ± 3.6; $P = 0.946$ for PEAK vs. START, $P = 0.007$ for time × KE effect, $r^2 = 0.36$). A time × KE effect ($P < 0.001$, $\eta_p^2 = 0.52$) showed that blood [glucose] (Fig. 4*B*) was ~1 mM lower in KE versus CON both at START and END WU ($P = 0.002$ and $P = 0.013$ for KE vs. CON, resp.), whereas [glucose] was similar

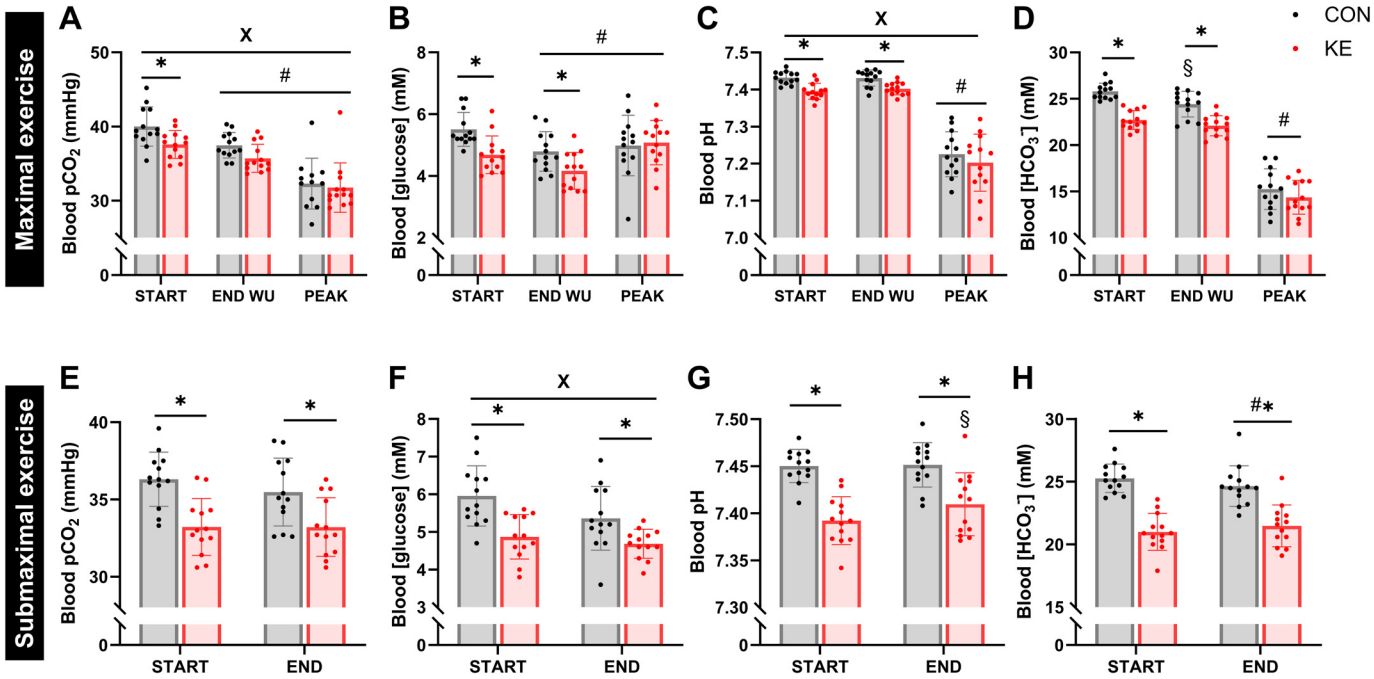

Figure 4. Effect of ketone ester (KE) ingestion on capillary blood pCO_2, glucose concentration, and acid-base balance during exercise. Data are represented as means ± SD along with individual values ($n = 13$) for capillary pCO_2, [glucose], pH, and [bicarbonate] measured in capillary blood samples during (*A–D*, resp.) maximal exercise, as well as (*E–H*, resp) submaximal exercise. During a 5.5-h normobaric hypoxic protocol involving intermittent KE (red) or placebo (CON, black) ingestion, participants completed a graded maximal exercise test after 1.5 h and a 30-min submaximal exercise bout after 3 h in hypoxia. Capillary blood samples were obtained before the start (START), during the final minute of warm-up (END WU), and immediately upon exhaustion (PEAK) of the maximal exercise, as well as before the start (START), and during the final minute (END) of the submaximal exercise. §$P < 0.05$ vs. START for indicated condition; #$P < 0.05$ vs. START for both conditions; X$P < 0.05$ for main KE effect; *$P < 0.05$ KE vs. CON for indicated timepoints.

between KE and CON upon PEAK ($P = 0.955$ for KE vs. CON). Blood pH (Fig. 4*C*) was unaffected throughout WU ($P = 0.993$ for END WU vs. START); however, it dropped in both conditions upon PEAK ($P < 0.001$ for PEAK vs. END WU; $P < 0.001$ for main time effect, $\eta_p^2 = 0.98$). Moreover, pH was ~0.03 units lower in KE compared with CON throughout EX_{MAX} ($P = 0.001$ for main KE effect, $\eta_p^2 = 0.50$, START: $P = 0.005$, END WU: $P = 0.030$, PEAK: $P = 0.111$ for KE vs. CON). [HCO_3^-] was ~3 mM lower in KE than in CON at START ($P < 0.001$ for KE vs. CON) and ~2 mM at END WU ($P < 0.001$ for KE vs. CON), whereas upon PEAK, [HCO_3^-] was ~15 mM in both KE and CON ($P = 0.103$ for KE vs. CON; $P = 0.002$ for time × KE effect, $\eta_p^2 = 0.40$). Blood [lactate] was stable at ~2.5 mM throughout WU ($P = 0.625$ for END WU vs. START, and $P > 0.784$ for KE vs. CON at START and END WU). Upon PEAK, [lactate] increased in both conditions ($P < 0.001$ vs. END WU) but remained lower in KE (13.2 ± 2.6 mM) than in CON (15.9 ± 4.0 mM, $P < 0.001$ for KE vs. CON, $P = 0.003$ for time × KE effect, $\eta_p^2 = 0.38$).

Submaximal exercise bout.

Throughout EX_{SUBMAX}, a time × KE effect was observed for pCO_2 ($P = 0.019$, $\eta_p^2 = 0.38$, Fig. 4*E*). Compared with CON, KE decreased pCO_2 by ~3 mmHg at START ($P < 0.001$ for KE vs. CON), and only by ~2 mmHg at END ($P < 0.001$ for KE vs. CON). Capillary pO_2 was consistently higher in KE compared with CON; however, the difference decreased from ~6 mmHg at START (CON: 40.9 ± 3.0 mmHg, KE: 46.3 ± 3.2 mmHg, $P = 0.001$ for KE vs. CON) to ~3 mmHg at END (CON: 34.6 ± 3.1 mmHg, KE: 37.1 ± 1.7 mmHg, $P = 0.014$ for KE vs. CON, $P = 0.004$ for time × KE effect, $r^2 = 0.57$). Blood p50 was also higher in KE versus CON both at START (CON: 24.7 ± 1.0 mmHg, KE: 26.3 ± 0.9 mmHg, $P < 0.001$ for KE vs. CON) and END (CON: 25.4 ± 0.8 mmHg, KE: 26.1 ± 0.9 mmHg, $P = 0.003$ for KE vs. CON). Whereas p50 increased throughout EX_{SUBMAX} in CON ($P = 0.004$ for END vs. START), values remained stable in KE ($P = 0.565$ for END vs. START; $P < 0.001$ for time × KE effect, $\eta_p^2 = 0.74$). The difference between pO_2 and p50 was higher throughout EX_{SUBMAX} in KE (START: 20.0 ± 3.6 mmHg, END: 10.9 ± 2.5 mmHg) compared with CON (START: 16.3 ± 3.0 mmHg, END: 9.3 ± 3.3 mmHg, $P = 0.013$ for main KE effect, $r^2 = 0.42$, START: $P = 0.017$, and END: $P = 0.093$ for KE vs. CON). Blood [glucose] remained stable during EX_{SUBMAX} in both conditions ($P = 0.062$ for main time effect), yet concentrations were consistently ~0.9 mM lower in KE versus CON ($P < 0.001$ for main KE effect, $\eta_p^2 = 0.73$, $P = 0.005$ for both for KE vs. CON, Fig. 4*F*). Blood pH was lower in KE (Fig. 4*G*) both at START (CON: 7.45 ± 0.02, KE: 7.39 ± 0.03, $P < 0.001$ for KE vs. CON) and END (CON: 7.45 ± 0.02, KE: 7.41 ± 0.03, $P < 0.001$ for KE vs. CON); however, values remained stable throughout EX_{SUBMAX} in CON ($P = 0.932$ for END vs. START) while increasing in KE ($P < 0.001$ for END vs. START; $P = 0.005$ for time × KE effect, $\eta_p^2 = 0.12$). Similarly, KE lowered blood [HCO_3^-] at START (CON: 25.3 ± 1.1 mM, KE: 21.0 ± 1.5 mM, $P < 0.001$ for KE vs. CON) and END (CON: 24.6 ± 1.6 mM, KE: 21.5 ± 1.7 mM, $P < 0.001$ for KE vs. CON), and whereas values dropped throughout EX_{SUBMAX} in CON ($P = 0.012$ for END vs. START), they increased in KE ($P = 0.047$ for END vs. START; $P = 0.001$ for time × KE effect, $\eta_p^2 = 0.59$).

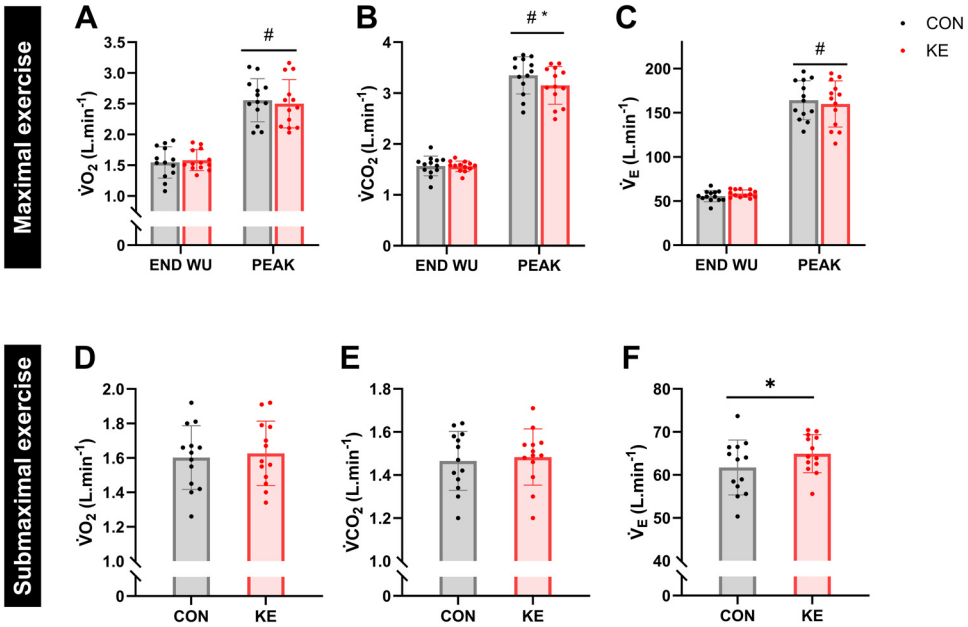

Figure 5. Effect of ketone ester (KE) ingestion on ventilatory parameters. Data are represented as means ± SD along with individual values ($n = 13$) for oxygen consumption rate ($\dot{V}O_2$), carbon dioxide production rate ($\dot{V}CO_2$), and ventilation ($\dot{V}E$) during (*A–C*, resp.) maximal exercise, as well as (*D–F*, resp.) submaximal exercise. During a 5.5-h normobaric hypoxic protocol involving intermittent KE (red) or placebo (CON, black) ingestion, participants completed a graded maximal exercise test after 1.5 h and a submaximal exercise bout after 3 h in hypoxia. Data are shown for the final 5 min of warm-up (END WU), and the final 30 s before exhaustion (PEAK) of the maximal exercise, as well as for the final 5 min of the submaximal exercise bout. #$P < 0.05$ vs. END WU for both conditions; *$P < 0.05$ KE vs. CON for indicated timepoints.

A time × KE effect was observed for blood [lactate] ($P < 0.001$, $\eta_p^2 = 0.79$). Blood lactate concentrations were higher in KE at START (CON: 2.3 ± 0.6 mM, KE: 2.8 ± 0.9 mM, $P = 0.002$ for KE vs. CON) but lower at END (CON: 2.8 ± 1.4 mM, KE: 2.2 ± 1.0 mM, $P < 0.001$ for KE vs. CON, $P < 0.001$ for time × KE effect, $\eta_p^2 = 0.79$).

KE Lowered $\dot{V}CO_2$ during Maximal Exercise and Induced Hyperventilation during Submaximal Exercise

Maximal exercise bout.

$\dot{V}O_2$ similarly increased in KE and CON throughout EX_{MAX} ($P < 0.001$ for main time effect, $\eta_p^2 = 0.99$; $P = 0.871$ for main KE effect, $\eta_p^2 = 0.01$; Fig. 5*A*). $\dot{V}CO_2$ was similar between KE and CON at END WU ($P = 0.994$ for KE vs. CON); however, values upon PEAK were lower in KE ($P = 0.010$ for KE vs. CON; $P = 0.036$ for time × KE effect, $\eta_p^2 = 0.32$, Fig. 5*B*). $\dot{V}E$ increased to a similar extent in both conditions from END WU to PEAK ($P < 0.001$ for main time effect, $\eta_p^2 = 0.99$; $P = 0.831$ for main KE effect; Fig. 5*C*), which was mediated by an increase in both breathing frequency (END WU: 26.1 ± 0.1 breaths·min^{-1}, PEAK: 59.2 ± 1.0 breaths·min^{-1}, $P < 0.001$ for main time effect, $\eta_p^2 = 0.99$) and tidal volume (END WU: 2.22 ± 0.08 L, PEAK: 3.12 ± 0.02 L, $P < 0.001$ for main time effect, $\eta_p^2 = 0.97$).

Submaximal exercise bout.

At the end of EX_{SUBMAX}, $\dot{V}O_2$ and $\dot{V}CO_2$ were similar between KE and CON ($P = 0.610$ and $P = 0.592$, Fig. 5, *D* and *E*, resp.). Conversely, $\dot{V}E$ was 5% higher in KE versus CON ($P = 0.036$, $g = 0.59$, Fig. 5*F*). This was established through a higher tidal volume (KE: 2.21 ± 0.36 L, CON: 2.03 ± 0.32, $P = 0.048$, $g = 0.52$) as breathing frequencies remained similar (KE: 30.2 ± 4.0 breaths·min^{-1}, CON: 31.3 ± 5.2 breaths·min^{-1}, $P = 0.381$).

KE Did Not Affect the Increase in Blood Erythropoietin Concentrations after 5 h in Hypoxia

A main time effect was observed for blood EPO concentrations ($P < 0.001$, $\eta_p^2 = 0.88$, Fig. 6), where EPO concentrations increased from 3.3 ± 0.5 IU·L^{-1} at baseline to 5.2 ± 0.4 IU·L^{-1} after 5 h (+56%). Blood EPO concentrations were not affected by KE ($P = 0.124$ for main KE effect; $P = 0.891$ for time × KE effect).

Blinding of Supplementation

After their second experimental session, participants attempted to identify the supplements they received during both sessions and indicated how confident they were in this choice through a continuous score between 0% (no idea at all) to 100% (completely certain). Within the 14 participants that were included in the larger project (7), eight participants were 0%–50% sure (and 6 guessed right) and six participants were 80%–90% sure (and guessed right). However, they also indicated that these decisions were based on their

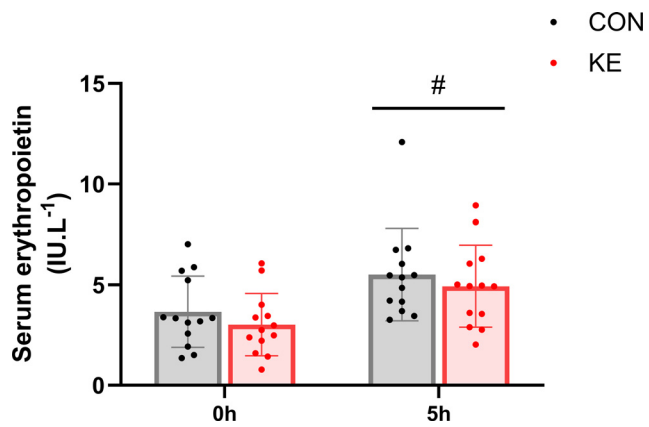

Figure 6. Effect of ketone ester (KE) ingestion on circulating erythropoietin and dopamine concentrations. Data are represented as means ± SD along with individual values ($n = 13$) for serum erythropoietin concentrations, determined using enzyme-linked immunosorbent assays (ELISAs). During a 5.5-h normobaric hypoxic protocol involving intermittent KE (red) or placebo (CON, black) ingestion, venous blood samples were collected before hypoxic entry (0 h) and after 5 h in hypoxia. #$P < 0.05$ vs. 0 h for both conditions.

perceived AMS symptoms during each session, as they expected to feel better upon KE [which was generally correct (7)]. Indeed, all participants that were 80%–90% sure endured the protocol longer in their KE versus CON session, and the participants with no/low AMS all indicated to be 0%–50% sure.

DISCUSSION

We aimed to determine the impact of KE on exercise performance and cognitive function upon acute hypoxic exposure, and to identify whether KE may further augment EPO concentrations after 5 h in hypoxia. Therefore, physically active participants were acutely exposed to a simulated altitude of 4,000 m during which exercise performance, cognitive function, and serum EPO were evaluated within the first 5.5 h upon hypoxic exposure. Our results show that KE did not impact cognitive function or serum EPO concentrations. However, KE impaired high-intensity exercise performance in hypoxia by ∼3.5%.

Despite the presence of contradictory findings, the current consensus seems to suggest that exogenous ketosis impairs high-intensity endurance exercise performance (18–21). To our knowledge, only one earlier report from our laboratory investigated the effect of KE supplementation on hypoxic high-intensity endurance performance (9). These data suggest an interesting perspective as any KE-induced ergolytic effect may be compensated by a KE-induced elevation in blood oxygenation, which has repeatedly been observed during exercise (9, 10) and rest (7, 8) in hypoxia. Nevertheless, this remains to be confirmed in various ecologically valid circumstances, and the effect on hypoxic high-intensity endurance performance at a timepoint where oxygenation is not (yet) elevated by KE, is to be elucidated. Our study protocol proved successful in evaluating this, given that blood oxygen saturation was similar (∼83%) between KE and CON at the start of EX_{MAX} (i.e., 2 h following the first KE dosage and 1.5 h following hypoxic entry). We observed that KE ingestion impaired exercise performance, as evidenced by a reduction of ∼10 W or 3.5% in achieved peak power during a graded exercise test. This aligns with earlier findings in normoxia reporting exogenous ketosis-induced reductions in peak power output of ∼4% in graded exercise performance tests (19), but also a reduction of ∼2% in average power output during a 30-min time trial (18) and a 7% and 2% reduction in fixed-distance 150 kJ (∼10-km) and 31-km time trials, respectively (20, 21). Moreover, this supports our hypothesis that the "intrinsic" ergolytic effect of KE ingestion on high-intensity exercise performance is only compensated in hypoxia whenever KE alleviates hypoxemia. Similar to normoxia, the observed ergolytic effect of KE in our study most likely resulted from ketoacidosis and/or impaired glycolytic energy production. This is supported by the lower blood pH (∼−0.03 units), [HCO_3^-] (∼−2 mM), as well as blood glucose and lactate concentrations throughout EX_{MAX} in KE versus CON.

Although blood oxygenation was not affected during EX_{MAX}, muscular oxygenation was already increased by KE. This is in line with our earlier research, where KE elevated muscular oxygenation ∼30 min before an increase in Sp_{O_2} was observed during a 3-h submaximal exercise bout in gradually increasing hypoxia (1,000 to 3,000 m) (9). Within that previous study, we indicated that this reflects a KE-induced reduction in muscular oxygen extraction, which potentially resulted from an increase in mechanical work-to-O_2 ratio upon KE. Based on our data, we hypothesize that this timing discrepancy between arterial and muscular oxygenation relates to the ketoacidosis-induced increase in p50 that was already evident during EX_{MAX}. p50 defines the arterial pO_2 corresponding to an oxygen saturation of 50%. Hence, the KE-induced increase in p50 indicates that KE caused a right shift of the sigmoidal oxyhemoglobin dissociation curve (ODC), resulting in lower Sp_{O_2} values for a given pO_2. However, KE also increased capillary pO_2 (likely via acidosis-induced hyperventilation). Although we acknowledge the discrepancies between capillary and arterial pO_2, these results are in line with previous literature indicating an increase in arterial, capillary, and hippocampal oxygenation upon ketosis (7, 9, 11, 35, 36). Interestingly, the KE-induced increase in pO_2 (∼ + 3 mmHg) was greater than the increase in p50 (∼ + 1 mmHg) during EX_{MAX}. This indicates that KE ingestion increased blood pO_2 relative to the "position" of the ODC resulting in a net increase in Sp_{O_2}. Consequently, the fact that KE primarily increases Sp_{O_2} when dropping below ∼85%, as well as the discrepancies between muscular and blood oxygenation could be explained by the sigmoidal nature of the ODC. Under "normal" physiological conditions and at Sp_{O_2} values above ∼85%, we are generally positioned within the "flat" part at the right-end side of the ODC, and a KE-induced increase in pO_2 will barely affect Sp_{O_2}. However, lower Sp_{O_2} values are positioned within the steep part of the ODC, and even small changes in pO_2 (relative to the position of the ODC, i.e., the p50) will have a large impact on Sp_{O_2}. Although this hypothesis holds true during resting conditions, an exercise-induced acidosis and drop in pO_2 may diminish the relative KE-induced right shift. This, together with the inability of KE to increase minimal pO_2 at PEAK, may have dampened this effect during EX_{MAX}. As such, Sp_{O_2} during EX_{MAX} may have been unaffected by KE ingestion despite its alleged location within the steep part of the ODC, which highlights the delicate interplay between the location on the ODC and the relative differences in pO_2 between conditions. However, given that the pO_2 is lower in muscle capillaries compared with arterial blood (37), and exercise exacerbates skeletal muscle acidosis and hypercapnia (38), increased pO_2 values earlier translate in higher muscular oxygenation than higher Sp_{O_2}.

During EX_{SUBMAX} (i.e., 3 h following hypoxic entry), Sp_{O_2} had dropped to ∼75% in both conditions, yet was 1%–2% higher in KE relative to CON. Concurrent p50 and pO_2, as well as the increase in pO_2 relative to the p50 were higher upon KE, therefore confirming the large impact of subtle pO_2-changes on Sp_{O_2} within the steep part of the ODC. When circling back to the ecological validity of our findings, the slight elevation in pO_2 and muscular oxygenation during EX_{MAX} appears insufficient to improve exercise performance. Nevertheless, these insights might prove valuable in other circumstances, such as medical conditions related to severe hypoxemia or muscular deoxygenation like COVID-19 (39), chronic obstructive pulmonary disease (COPD) (40), or pulmonary embolization (41).

Our data showed no beneficial effects of KE on hypoxic cognitive function. This may seem to conflict with the earlier

observed dampened drop in cognitive efficiency (8). However, all other cognitive parameters that were assessed within that earlier study [i.e., 7 parameters in a Defense Automated Neurobehavioral Assessment (DANA), and 4 parameters in a RightEye oculometric assessment], including reaction time, remained unaffected by KE. Moreover, their hyper-acute protocol (i.e., start of cognitive assessment after 5 min at FI_{O_2}: 9.7%), and thus, different hypoxic doses hinder appropriate comparison. As such, the limited data available today does not provide clear support that KE can mitigate acute hypoxia- or exercise-induced disruptions in cognitive functioning, let alone improve cognitive function in hypoxia. The lack of cognitive improvement upon KE ingestion in hypoxia may be related to cerebral blood flow, which is considered a primary mechanism by which ketone bodies may enhance cognitive function (26). As we previously reported (7), KE increased resting cerebral oxygenation after 4 h in this study. This increased cerebral oxygenation would be expected to result in improved cognitive function (42). However, this occurred together with a reduction in cerebral blood flow, as well as cerebral oxygen delivery, which may have counteracted such effect as extensive research confirmed the importance of both cerebral blood flow and oxygenation in terms of cognition (42, 43). Furthermore, the negative effects of hypoxia on cognitive function are also mediated by various factors of which it is currently unknown if they are affected by KE. These include among others cerebral lactate accumulation, neuroinflammation, calcium overload, and increased reactive oxygen species (44).

Despite the recently identified ability of KE to increase postexercise circulating erythropoietin (EPO) concentrations in normoxia, the effect in hypoxia remained unexplored. As expected, 5 h of exposure to a simulated altitude of 4,000 m combined with maximal and submaximal exercise in our study drastically augmented ($\sim +56\%$) circulating EPO concentrations. Nevertheless, the implemented study design does not allow us to identify the relative contributions of hypoxia and/or exercise to this increase in [EPO]. Given that both sessions only differed by the ingested supplements, these data clearly indicate that KE was ineffective to further augment this response. However, the underlying mechanism or any interactions between KE and hypoxia/exercise remain unknown. This inability of KE to augment [EPO] most likely resulted from the higher oxygen saturation in KE versus CON that emerged after $\sim$3 h in hypoxia. The primary stimulus for EPO production is the reduced pO_2 within the renal cortex (30). Until today, there has been no data available on the effect of KE on renal pO_2. However, we can only assume that the KE-related increase in blood, brain, and skeletal muscle oxygenation status, as well as capillary pO_2 values in our study, would also translate into an increased renal pO_2, and thereby result in an "impaired" EPO stimulus. In this context, the alleviation of hypoxemia may very well protect against performance impairments and AMS yet may inadvertently hinder other potentially beneficial physiological adaptations as "collateral damage." Although this interaction may contribute to the inability of KE to increase EPO in our study, an alternative explanation may be related to the mechanism by which KE has been hypothesized to elevate EPO in normoxia. This is believed to result from a βHB-induced upregulation of histone H3 lysine 9 (H3K9) acetylation in kidney cells (45). Similar to βHB, hypoxia also induces such acetylation of H3K9 (46), thereby potentially leveling out the effect of βHB on H3K9. Alternatively, while βHB may indeed stimulate H3K9 acetylation, the KE-induced increase in oxygenation could diminish the hypoxia-induced upregulation. Finally, we respect the possibility that the stimulus provided by systemic hypoxia (i.e., induced by hypoxia, exercise, and/or a combined effect) simply overrules more subtle alternative regulatory mechanisms.

While this research provides interesting insights and conclusions, careful interpretation is warranted due to several limitations. First, only males were recruited for participation given the debated and ambiguous role of the hormonal cycle on, for example, exercise performance (47), cognition (48), and emotional-cognitive interplay (49), as well as menstruation-related discomfort potentially influencing AMS scoring in the original research project (7). Therefore, it remains to be identified whether similar effects would also be observed in female individuals. Second, our study was performed in normobaric hypoxia. Although cognitive function and EPO are considered to be similar in normobaric versus hypobaric hypoxia (50–52), cycling performance may be impaired to a greater extent in hypobaric hypoxia (53). Moreover, the lower blood glucose concentrations in the KE group at START and END WU of EX_{MAX} and START and END of EX_{SUBMAX} most likely resulted from a KE-induced reduction in hepatic glucogenesis (54, 55). But, given the increased reliance on glucose during exercise in hypoxia, it cannot be excluded that the lower blood glucose observed at the start of EX_{MAX} contributed to the impaired exercise performance upon KE. Nevertheless, [GLU] at the end of EX_{MAX} (i.e., PEAK) was similar between KE and CON, and well above the hypoglycemic threshold of $\sim$3.9 mM, opposing that low blood [GLU] contributed to the impaired performance in KE. Finally, normoxic baseline values were not evaluated, nor was a normoxic session included to isolate the independent effects of exercise versus hypoxia on cognitive function or EPO production. Nevertheless, we did not specifically aim to evaluate any hypoxia-/exercise-induced responses, nor to identify the relative contributions and underlying mechanisms, but rather whether KE may substantially alter these parameters at a given timepoint compared with CON ingestion.

In conclusion, the present work demonstrates that KE ingestion impairs high-intensity endurance performance in acute hypoxia, at least when blood oxygenation has not been increased by KE. Moreover, we propose that the mixed temporal effect of KE on blood and tissue oxygenation is related to the intimate interplay between pO_2, p50, and the position in the sigmoidal oxyhemoglobin dissociation curve. KE ingestion was also ineffective in ameliorating cognitive function in hypoxia, despite an increased blood or cerebral oxygenation status. Finally, despite promising data in normoxia, we are the first to show that KE does not upregulate the hypoxia-induced increase in circulating EPO concentrations during the first few hours of exposure. Taken together, the present data do not support the usage of KE supplements in acute hypoxia, except to alleviate the development of acute mountain sickness.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

The authors thank all participants and students for the dedicated cooperation in this study. We also thank Ms. Monique Ramaekers for her skillful assistance during the experimental trials.

GRANTS

This research was supported by the Research Foundation – Flanders (FWO Weave, Research Grant G073522N) and Slovene Research Agency Grant N5-0247. C.P. is supported by an FWO postdoctoral research Grant 12B0E24N.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.S., T.D., and C.P. conceived and designed research; M.S., D.T., W.L., R.R., M.R., and T.D. performed experiments; M.S. and T.D. analyzed data; M.S., T.D., and C.P. interpreted results of experiments; M.S. prepared figures; M.S. drafted manuscript; M.S., T.D., and C.P. edited and revised manuscript; M.S., D.T., W.L., R.R., M.R., T.D., and C.P. approved final version of manuscript.

REFERENCES

1. **Wehrlin JP**, **Hallén J.** Linear decrease in VO2max and performance with increasing altitude in endurance athletes. *Eur J Appl Physiol* 96: 404–412, 2006. doi:10.1007/s00421-005-0081-9.
2. **Steinman Y**, **Groen E**, **Frings- Dresen MHW.** Hypoxia impairs reaction time but not response accuracy in a visual choice reaction task. *Appl Ergon* 113: 104079, 2023. doi:10.1016/j.apergo.2023.104079.
3. **Blacker KJ**, **Seech TR**, **Funke ME**, **Kinney MJ.** Deficits in visual processing during hypoxia as evidenced by visual mismatch negativity. *Aerosp Med Hum Perform* 92: 326–332, 2021. doi:10.3357/AMHP.5735.2021.
4. **Malle C**, **Quinette P**, **Laisney M**, **Bourrilhon C**, **Boissin J**, **Desgranges B**, **Eustache F**, **Piérard C.** Working memory impairment in pilots exposed to acute hypobaric hypoxia. *Aviat Space Environ Med* 84: 773–779, 2013. doi:10.3357/asem.3482.2013.
5. **Gallagher SA**, **Hackett PH.** High-altitude illness. *Emerg Med Clin North Am* 22: 329–355, viii, 2004. doi:10.1016/j.emc.2004.02.001.
6. **West JB.** High-altitude medicine. *Am J Respir Crit Care Med* 186: 1229–1237, 2012. doi:10.1164/rccm.201207-1323CI.
7. **Stalmans M**, **Tominec D**, **Lauriks W**, **Robberechts R**, **Debevec T**, **Poffé C.** Exogenous ketosis attenuates acute mountain sickness and mitigates high-altitude hypoxemia. *J Appl Physiol (1985)* 137: 1301–1312, 2024. doi:10.1152/japplphysiol.00190.2024.
8. **McClure TS**, **Phillips J**, **Koutnik AP**, **Coleman K**, **Chappe E**, **Cutter GR**, **Egan B**, **Norell T**, **Stubbs BJ**, **Bamman MM**, **Kernagis D.** Ketone monoester attenuates declines in cognitive performance and oxygen saturation during acute severe hypoxic exposure under resting conditions. *Exp Physiol* 109: 1672–1682, 2024. doi:10.1113/EP091794.
9. **Poffé C**, **Robberechts R**, **Podlogar T**, **Kusters M**, **Debevec T**, **Hespel P.** Exogenous ketosis increases blood and muscle oxygenation but not performance during exercise in hypoxia. *Am J Physiol Regul Integr Comp Physiol* 321: R844–R857, 2021. doi:10.1152/ajpregu.00198.2021.
10. **McClure TS**, **Phillips J**, **Kernagis D**, **Coleman K**, **Chappe E**, **Cutter GR**, **Egan B**, **Norell T**, **Stubbs BJ**, **Bamman MM**, **Koutnik AP.** Ketone monoester attenuates oxygen desaturation during weighted ruck exercise under acute hypoxic exposure but does not impact cognitive performance. *Exp Physiol* 109: 1768–1781, 2024. doi:10.1113/EP091789.
11. **Stalmans M**, **Tominec D**, **Robberechts R**, **Lauriks W**, **Ramaekers M**, **Debevec T**, **Poffé C.** A Single Night in Hypoxia Either with or without Ketone Ester Ingestion Reduces Sleep Quality without Impacting Next-Day Exercise Performance. *Med Sci Sports Exerc* 57: 807–819, 2025. doi:10.1249/MSS.0000000000003604.
12. **Cox PJ**, **Kirk T**, **Ashmore T**, **Veech RL**, **Griffin JL**, **Clarke K**, **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. **Brooks E**, **Lamothe G**, **Nagpal TS**, **Imbeault P**, **Adamo K**, **Kara J**, **Doucet E.** Acute ingestion of ketone monoesters and precursors do not enhance endurance exercise performance: a systematic review and meta-analysis. *Int J Sport Nutr Exerc Metab* 32: 214–225, 2022. doi:10.1123/ijsnem.2021-0280.
14. **Valenzuela PL**, **Castillo-García A**, **Morales JS**, **Lucia A.** Perspective: ketone supplementation in sports-does it work? *Adv Nutr* 12: 305–315, 2021. doi:10.1093/advances/nmaa130.
15. **Sun K**, **Choi YT**, **Yu CCW**, **Nelson EAS**, **Goh J**, **Dai S**, **Hui LL.** The effects of ketogenic diets and ketone supplements on the aerobic performance of endurance runners: a systematic review. *Sports Health* 19417381241271547, 2024. doi:10.1177/19417381241271547.
16. **Valenzuela PL**, **Morales JS**, **Castillo-García A**, **Lucia A.** Acute ketone supplementation and exercise performance: a systematic review and meta-analysis of randomized controlled trials. *Int J Sports Physiol Perform* 15: 298–308, 2020. doi:10.1123/ijspp.2019-0918.
17. **Margolis LM**, **O'Fallon KS.** Utility of ketone supplementation to enhance physical performance: a systematic review. *Adv Nutr* 11: 412–419, 2020. doi:10.1093/advances/nmz104.
18. **Poffé C**, **Wyns F**, **Ramaekers M**, **Hespel P.** Exogenous ketosis impairs 30-min time-trial performance independent of bicarbonate supplementation. *Med Sci Sports Exerc* 53: 1068–1078, 2021. doi:10.1249/MSS.0000000000002552.
19. **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.
20. **Leckey JJ**, **Ross ML**, **Quod M**, **Hawley JA**, **Burke LM.** Ketone diester ingestion impairs time-trial performance in professional cyclists. *Front Physiol* 8: 806–810, 2017. doi:10.3389/fphys.2017.00806.
21. **O'Malley T**, **Myette-Cote E**, **Durrer C**, **Little JP.** Nutritional ketone salts increase fat oxidation but impair high-intensity exercise performance in healthy adult males. *Appl Physiol Nutr Metab* 42: 1031–1035, 2017. doi:10.1139/apnm-2016-0641.
22. **Ramos-Campo DJ**, **López-Román FJ**, **Pérez-Piñero S**, **Ortolano R**, **Abellán-Ruiz MS**, **de los Cobos EMP**, **Luque-Rubia AJ**, **Van Elslande D**, **Ávila-Gandía V.** Effects of ketone monoester and bicarbonate co-ingestion on cycling performance in WorldTour Cyclists. *Int J Sport Nutr Exerc Metab* 34: 1–10, 2024. doi:10.1123/ijsnem.2023-0078.
23. **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.
24. **McMorris T**, **Hale BJ**, **Barwood M**, **Costello J**, **Corbett J.** Effect of acute hypoxia on cognition: a systematic review and meta-regression analysis. *Neurosci Biobehav Rev* 74: 225–232, 2017 [Erratum in *Neurosci Biobehav Rev* 98:333, 2019]. doi:10.1016/j.neubiorev.2017.01.019.
25. **Fowler B**, **Taylor M**, **Porlier G.** The effects of hypoxia on reaction time and movement time components of a perceptual-motor task. *Ergonomics* 30: 1475–1485, 1987. doi:10.1080/00140138708966040.
26. **Walsh JJ**, **Caldwell HG**, **Neudorf H**, **Ainslie PN**, **Little JP**, **Barrett K**, **Rickards C**, **Walsh JJ.** Short-term ketone monoester supplementation improves cerebral blood flow and cognition in obesity: a randomized cross-over trial. *J Physiol* 599: 4763–4778, 2021. doi:10.1113/JP281988.

27. **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.
28. **Prins PJ**, **D'Agostino DP**, **Rogers CQ**, **Ault DL**, **Welton GL**, **Jones DW**, **Henson SR**, **Rothfuss TJ**, **Aiken KG**, **Hose JL**, **England EL**, **Atwell AD**, **Buxton JD**, **Koutnik AP.** Dose response of a novel exogenous ketone supplement on physiological, perceptual and performance parameters. *Nutr Metab (Lond)* 17: 1–12, 2020. doi:10.1186/s12986-020-00497-1.
29. **Poffé C**, **Robberechts R**, **Stalmans M**, **Vanderroost J**, **Bogaerts S**, **Hespel P.** Exogenous ketosis increases circulating dopamine concentration and maintains mental alertness in ultra-endurance exercise. *J Appl Physiol (1985)* 134: 1456–1469, 2023. doi:10.1152/japplphysiol.00791.2022.
30. **Jelkmann W.** Erythropoietin: structure, control of production, and function. *Physiol Rev* 72: 449–489, 1992. doi:10.1152/physrev.1992.72.2.449.
31. **Evans E**, **Walhin JP**, **Hengist A**, **Betts JA**, **Dearlove DJ**, **Gonzalez JT.** Ketone monoester ingestion increases postexercise serum erythropoietin concentrations in healthy men. *Am J Physiol Endocrinol Physiol* 324: E56–E61, 2023. doi:10.1152/ajpendo.00264.2022.
32. **Poffé C**, **Robberechts R**, **Thienen R**, **Van Hespel P.** Exogenous ketosis elevates circulating erythropoietin and stimulates muscular angiogenesis during endurance. *J Physiol* 601: 2345–2358, 2023. doi:10.1113/JP284346.
33. **Burtscher M.** Endurance performance of the elderly mountaineer: requirements, limitations, testing, and training. *Wien Klin Wochenschr* 116: 703–714, 2004. doi:10.1007/s00508-004-0258-y.
34. **Nation DA**, **Bondi MW**, **Gayles E**, **Delis DC.** Mechanisms of memory dysfunction during high altitude hypoxia training in military aircrew. *J Int Neuropsychol Soc* 23: 1–10, 2017. doi:10.1017/S1355617716000965.
35. **Gom RC**, **Bhatt D**, **Villa BR**, **George AG**, **Lohman AW**, **Mychasiuk R**, **Rho JM**, **Teskey GC.** The ketogenic diet raises brain oxygen levels, attenuates postictal hypoxia, and protects against learning impairments. *Neurobiol Dis* 154: 105335, 2021. doi:10.1016/j.nbd.2021.105335.
36. **Zhang K**, **Zhang H**, **Yi H**, **Huang G**, **Zhao X**, **Yu S**, **Xu W.** The protective effects of 1,3-butanediol acetoacetate diester on decompression sickness in rats. *J Appl Physiol (1985)* 131: 435–441, 2021. doi:10.1152/japplphysiol.00035.2021.
37. **Richardson RS**, **Duteil S**, **Wary C**, **Wray DW**, **Hoff J**, **Carlier PG.** Human skeletal muscle intracellular oxygenation: the impact of ambient oxygen availability. *J Physiol* 571: 415–424, 2006. doi:10.1113/jphysiol.2005.102327.
38. **Robergs RA**, **Ghiasvand F**, **Parker D.** Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol* 287: R502–R516, 2004. doi:10.1152/ajpregu.00114.2004.
39. **Swenson KE**, **Hardin CC.** Pathophysiology of hypoxemia in COVID-19 lung disease. *Clin Chest Med* 44: 239–248, 2023. doi:10.1016/j.ccm.2022.11.007.
40. **Kent BD**, **Mitchell PD**, **Mcnicholas WT.** Hypoxemia in patients with COPD: cause, effects, and disease progression. *Int J Chron Obstruct Pulmon Dis* 6: 199–208, 2011. doi:10.2147/COPD.S10611.
41. **Altemeier WA**, **Robertson HT**, **Mckinney S**, **Glenny RW.** Pulmonary embolization causes hypoxemia by redistributing regional blood flow without changing ventilation. *J Appl Physiol (1985)* 85: 2337–2343, 1998. doi:10.1152/jappl.1998.85.6.2337.
42. **Williams TB**, **Corbett J**, **McMorris T**, **Young JS**, **Dicks M**, **Ando S**, **Thelwell RC**, **Tipton MJ**, **Costello JT.** Cognitive performance is associated with cerebral oxygenation and peripheral oxygen saturation, but not plasma catecholamines, during graded normobaric hypoxia. *Exp Physiol* 104: 1384–1397, 2019. doi:10.1113/EP087647.
43. **Ogoh S.** Relationship between cognitive function and regulation of cerebral blood flow. *J Physiol Sci* 67: 345–351, 2017. doi:10.1007/s12576-017-0525-0.
44. **Wang X**, **Cui L**, **Ji X.** Cognitive impairment caused by hypoxia: from clinical evidences to molecular mechanisms. *Metab Brain Dis* 37: 51–66, 2022. doi:10.1007/s11011-021-00796-3.
45. **Robberechts R**, **Poffé C.** Defining ketone supplementation: the evolving evidence for post-exercise ketone supplementation to improve recovery and adaptation to exercise. *Am J Physiol Cell Physiol* 326: C143–C160, 2024. doi:10.1152/ajpcell.00485.2023.
46. **Steinmann K**, **Richter AM**, **Dammann RH.** Epigenetic silencing of erythropoietin in human cancers. *Genes Cancer* 2: 65–73, 2011. doi:10.1177/1947601911405043.
47. **Carmichael MA**, **Thomson RL**, **Moran LJ**, **Wycherley TP.** The impact of menstrual cycle phase on athletes' performance: a narrative review. *Int J Environ Res Public Health* 18: 1–24, 2021. doi:10.3390/ijerph18041667.
48. **Le J**, **Thomas N**, **Gurvich C.** Cognition, the menstrual cycle, and premenstrual disorders: a review. *Brain Sci* 10: 1–14, 2020. doi:10.3390/brainsci10040198.
49. **Sacher J**, **Okon-Singer H**, **Villringer A.** Evidence from neuroimaging for the role of the menstrual cycle in the interplay of emotion and cognition. *Front Hum Neurosci* 7: 374–377, 2013. doi:10.3389/fnhum.2013.00374.
50. **Mounier R**, **Brugniaux JV.** Counterpoint: Hypobaric hypoxia does not induce different responses from normobaric hypoxia. *J Appl Physiol (1985)* 112: 1784–1786, 2012. doi:10.1152/japplphysiol.00067.2012.
51. **Hohenauer E**, **Freitag L**, **Costello JT**, **Williams TB**, **Küng T**, **Taube W**, **Herten M**, **Clijsen R.** The effects of normobaric and hypobaric hypoxia on cognitive performance and physiological responses: a crossover study. *PLoS One* 17: e0277364, 2022. doi:10.1371/journal.pone.0277364.
52. **Aebi MR**, **Bourdillon N**, **Noser P**, **Millet GP**, **Bron D.** Cognitive impairment during combined normobaric vs. hypobaric and normoxic vs. hypoxic acute exposure. *Aerosp Med Hum Perform* 91: 845–851, 2020. doi:10.3357/AMHP.5616.2020.
53. **Beidleman BA**, **Fulco CS**, **Staab JE**, **Andrew SP**, **Muza SR.** Cycling performance decrement is greater in hypobaric versus normobaric hypoxia. *Extrem Physiol Med* 3: 8–9, 2014. doi:10.1186/2046-7648-3-8.
54. **Evans M**, **Cogan KE**, **Egan B.** Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation. *J Physiol* 595: 2857–2871, 2017. doi:10.1113/JP273185.
55. **Stubbs BJ**, **Cox PJ**, **Evans RD**, **Santer P**, **Miller JJ**, **Faull OK**, **Magor-Elliott S**, **Hiyama S**, **Stirling M**, **Clarke K.** On the metabolism of exogenous ketones in humans. *Front Physiol* 8: 848, 2017. doi:10.3389/fphys.2017.00848.