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Exogenous ketosis attenuates acute mountain sickness and mitigates normobaric high-altitude hypoxemia

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Running Head: Exogenous ketosis mitigates high-altitude hypoxemia.

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New & Noteworthy: Ketone ester intake attenuated the development of acute mountain sickness at a simulated altitude of 4,000-4,500m. This likely resulted from a mitigation of arterial and cerebral hypoxemia, reduced cerebral blood flow and increased sympathetic drive.

Keywords: ketones, hypoxia, acute mountain sickness (AMS), oxygen saturation, cerebral oxygenation.

ABSTRACT

Background: Acute mountain sickness (AMS) represents a considerable issue for individuals sojourning to high altitudes with systemic hypoxemia known to be intimately involved in its development. Based on recent evidence that ketone ester (KE) intake attenuates hypoxemia, we investigated whether exogenous ketosis might mitigate AMS development and to identify underlying physiological mechanisms. **Methods:** Fourteen healthy, male participants were enrolled in two 29h protocols (simulated altitude of 4,000-4,500m) receiving either KE or a placebo (CON) at regular timepoints throughout the protocol in a randomized, crossover manner. Physiological responses were characterized after 15min and 4h in hypoxia, and the protocol was terminated prematurely upon development of severe AMS (Lake Louise Score ≥ 10). **Results:** KE ingestion induced a consistent diurnal ketosis ([ßHB] of ~3 mM), whereas blood [ßHB] remained low (<0.6 mM) in CON. Each participant tolerated the protocol equally long or longer (n=6 or n=8, resp.) in KE. Protocol duration increased by 32% on average with KE, and doubled upon KE for severe AMS-developing participants (n=9). Relative to CON, KE induced a mild metabolic acidosis, hyperventilation, and relative sympathetic dominance. KE also inhibited the progressive hypoxemia that was observed between 15min and 4h in hypoxia in CON, while concomitantly increasing cerebral oxygenation and capillary pO_2 within this timeframe despite a KE-induced reduction in cerebral oxygen supply. **Conclusions:** These data indicate that exogenous ketosis attenuates AMS development. The key underlying mechanisms include improved arterial and cerebral oxygenation, in combination with lowered cerebral blood flow and oxygen delivery, and increased sympathetic dominance.

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INTRODUCTION

Acute mountain sickness (AMS) is the most prevalent form of altitude illness and develops in ~25% of individuals upon exposure to 2,500m altitude [1] with its prevalence further increasing at higher altitudes [2, 3]. It presents as a syndrome of nonspecific symptoms including headache, nausea, vomiting, dizziness or fatigue, eventually also compromising general functioning [1]. Although AMS is typically self-limiting and usually resolves within 2-3 days [4], it can progress into life-threatening conditions such as high-altitude pulmonary (HAPE) or cerebral (HACE) oedema [5]. Hypoxemia is considered to be the primary factor driving AMS pathogenesis [6], and for instance increases cerebral blood flow in order to preserve oxygen delivery to the brain which in turn may increase intracranial pressure [7, 8]. Despite a hypoxemia-induced initial stimulation of the sympathetic response[9], relative dominance of the parasympathetic nervous system was found to play a crucial role in AMS development [10]. This was found to be established through AMS-like symptoms arising from vagal (hyper-)activity [11, 12]. Some pharmacological interventions (*e.g.,* acetazolamide) have been shown to reduce AMS symptoms [13, 14]. However, a recent Cochrane review concluded that there is currently no clear evidence for any non-pharmacological intervention to robustly mitigate AMS [15].

Interestingly, early studies in rodents observed that increasing ketone bodies (KB) in the blood improved tolerance to extreme hypoxia (F_IO_2: 4-5%) [16, 17]. KB, especially acetoacetate (AcAc) and D-β-hydroxybutyrate (βHB), are fatty acid derived molecules that are primarily produced in the liver upon conditions of reduced carbohydrate availability, and have been shown to be preferentially utilized as an energy source by the brain under hypoxic stress in rats [18]. More recently, we observed that increasing blood ketone bodies via ketone ester (KE) ingestion also attenuated the drop in oxygen saturation in human participants

during exercise at ~2,500-3,000m simulated altitude [19]. This KE-induced attenuation of hypoxemia was primarily attributed to an increase in arterial pO_2 caused by ketoacidosis-induced hyperventilation. This may be of primary importance given the above mentioned direct relationship between the extent of hypoxemia and AMS susceptibility and severity [6].

Accordingly, these data indicate that increasing blood KB may be a useful strategy to mitigate the development of AMS. Against this background, we aimed to identify whether increasing blood KB via oral KE ingestion can inhibit the development and severity of AMS, as well as to determine the potential underlying physiological mechanism(s). We hypothesize that KE ingestion alleviates hypoxemia, at least after 3h in hypoxia, and therefore potentially postpones AMS development and mitigates associated symptoms.

METHODS

Study design and participants. This randomized, double-blind, placebo-controlled, crossover 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. We recruited healthy, male participants with the following inclusion criteria: (i) age: 18-35 years, (ii) body mass index (BMI): 18-25 $kg.m^{-2}$, (iii) regular physically active: 2-7 $h.week^{-1}$. Exclusion criteria included smoking, a history of pathologies that are considered a contra indication for high-altitude exposure (*e.g.* coronary artery disease, pulmonary disease, COPD, …), and pre-exposure to altitudes above 1,500m in the three months preceding the study. Despite the original plan to include female participants, a limited availability of the hypoxic facility did not allow for sufficient wash-out between both sessions in order to standardize hormonal balance across the cycle. All participants provided written informed consent before inclusion in the study. Allocation of the subjects to the experimental conditions was performed *at random*, yet stratified based on oxygen saturation after 30min at a simulated altitude of 4,000m. Subjects and researchers were blinded to the experimental conditions by using a placebo for KE similar in taste and appearance. At the level of the investigators, blinding was ensured by having the randomization done by a person who was otherwise not involved in the experimental testing. Blood ketone concentrations were assessed by an investigator who was neither involved in the randomization procedure nor in any of the other measurements.

As AMS typically peaks after 16-24h of hypoxic exposure [4], we designed a 29h protocol (Fig. 1) in a normobaric facility (for details, see [19]) with the explicit purpose to elicit AMS. Throughout both experimental sessions, separated by a 1-week washout period, participants received either ketone ester (KE) or placebo drinks (CON) in a counterbalanced order. During the first 26h of each session, the participants resided at a simulated altitude of

4,000m (F_IO_2: 12.7%), after which they were immediately transferred to 4,500m (F_IO_2: 11.8%). Throughout the protocol, participants regularly performed exercise bouts on a calibrated cycling ergometer (Avantronic Cyclus II, Leipzig, Germany) to simulate the workload associated with normal ascend rates [20] and to facilitate AMS development [21]. This immediate transition to high altitude is more abrupt than most high-altitude expeditions or competitions, however rather finds its validity in situations like high-altitude airports (e.g., La Paz, 4.061m), cable cars to > 3.000m viewpoints (e.g. Dagu Glacier Gondola, 4.860 m), and athletes sleeping in hypoxic chambers/tents. The protocol was terminated prematurely if the participant developed severe AMS [Lake Louise Score (LLS) of ≥ 10 out of 15 [1]], resulting in a distribution of (i) all participants (AMS_{all}) over (ii) AMS-sensitive participants that developed severe AMS leading to premature termination of the experimental protocol in at least one of both sessions (AMS_{high}), and (iii) participants that completed both sessions (AMS_{low}) without severe AMS development. Both experimental sessions started on the same time of the day within a given individual (between 7:00AM and 10:00AM). Based on previously performed pilot testing, the participants' tolerated time in hypoxia (*i.e.* before developing severe AMS) was defined as the primary outcome of the study.

Preliminary testing. One week before the first experimental session, participants engaged in a normoxic familiarization session in which they performed an incremental cycling test to determine their maximal oxygen consumption rate ($\dot{V}O_2max$, *e.g.,* highest value over a 15sec period) using indirect calorimetry (Cortex Metalyzer IIIb, Leipzig, Germany). After 15min of passive recovery, participants were familiarized with all the measurements that were performed during the experimental sessions. From their familiarization session until after their second session, participants were instructed to maintain a constant sleep-wake rhythm with at least 7 hours of sleep per night. In order not to deviate from their normal sleep-wake rhythm,

119 they chose the time of wake-up (between 7:00 and 9:00) and start of sleep (between 22:00 and
120 24:00) to be maintained constant throughout the study. Adherence to this sleep/wake schedule
121 was monitored throughout the entire study period using a sleep diary and an actigraphic
122 wristband (ActiGraph wGT3X- BT, Actigraphcorp, USA).
123
124 *Exercise bouts.* During the familiarization session, participants performed an incremental
125 test to determine their maximal oxygen uptake rate ($\dot{V}O_2max$) on a cycling ergometer
126 (Avantronic Cyclus II, Leipzig, Germany). After 10min of warming up at 70W, resistance
127 was set at 100W and increased by 10 W/30sec until voluntary exhaustion. The protocol
128 included four 30min submaximal exercise bouts (30min at 1.5W.kg^{-1}; performed after 3, 6, 7
129 and 8h at altitude), 2 maximal exercise bouts (10min at 1.5W.kg^{-1} followed by 100W +
130 10W/30sec until voluntary exhaustion; performed after 1.5 and 25h at altitude), and one
131 combined exercise bout (30min at 1.5W.kg^{-1} followed by 100W + 10W/30sec until voluntary
132 exhaustion; performed after 26.5h at altitude).
133
134 *Nutritional intervention.* In the KE condition, participants received the ketone monoester
135 (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (KetoneAid Inc., Falls Church, Virginia, USA;
136 ~4.7 kcal.g^{-1}) in boluses of either 25g (30min before entering hypoxia, as well as after 1, 4, 23
137 and 24.5h in hypoxia and 30min before sleep) or 12.5g (after 2.5, 5, 6.5, 7.5, 9, 26 and 27.5h
138 in hypoxia) with the purpose to establish a stable ketosis ([ßHB] ~2-5 mM) during the diurnal
139 parts of the protocol, which was previously tested in a pilot run. In CON, participants received
140 a non-isocaloric, taste and viscosity matched placebo consisting of 12.5% w/v collagen (6d
141 Sports Nutrition, Oudenaarde, Belgium) and 1 mM bitter sucrose octaacetate (Sigma-Aldrich,
142 Bornem, Belgium) dissolved in water to generate either supplements of 12.5g or 25g. The
143 bitter taste of both supplements was softened by adding 5% w/v sucralose (MyProtein, New

144 York City, US) and 1.0% v/v strawberry flavor drops (MyProtein, New York City, US). If
145 participants completed the full protocol, total caloric intake via the KE supplements was
146 ~1000 kcal *vs.* ~100 kcal for the CON supplements. Supplements were provided in
147 nontransparent 50 mL tubes to avoid potential visual identification.

148 *Dietary control.* Participants were provided, in sequential order (i) a carbohydrate-rich
149 dinner (~5,600 kJ; 69% carbohydrate, 16% fat, 15% protein) the evening before the
150 experimental session, (ii) a breakfast (~4,100 kJ; 64% carbohydrate, 26% fat, and 10%
151 protein) 30min before entering the hypoxic room, (iii) a lunch after 3.5h in hypoxia (~3,950
152 kJ; 53% carbohydrate, 26% fat, and 18% protein), (iv) a light evening meal after 10.5h in
153 hypoxia (~2,330 kJ; 67% carbohydrate, 5% fat, and 26% protein), and (v) an identical
154 breakfast on the same time of the day as on day 1. In addition, a carbohydrate-rich snack
155 (~660 kJ; 92% carbohydrate, 2% fat, and 4% protein) was provided after 2.5, 6.5, 9, 12 and
156 26.5 h in hypoxia. Participants were allowed to ask for more food during the first session if
157 they felt hungry, and additional caloric intake was then replicated during the second session.
158 Total energy intake was standardized (~4000 kcal during the 29h protocol excluding KE/CON
159 supplements), while water intake was *ad libitum* but replicated within a given individual, from
160 the evening before until the end of each 29h experimental session.
161
162 *Physiological measurements.* To characterize the resting physiological responses,
163 measurement bouts were performed after 15min and 4h of hypoxic exposure. This included
164 sequential determination of (i) blood oxygen saturation (SpO_2), (ii) cerebral (prefrontal
165 cortex) and skeletal muscle (*m. vastus lateralis*) oxygenation status, (iii) cerebral blood flow
166 through the internal carotid artery (ICA) and the vertebral artery (VA), which constitute the
167 main intracranial blood supply [19] and, concurrently, oxygen delivery calculated as
168 (VA+ICA)*SpO_2 [22], (iv) respiratory gas exchange, (v) heart rate and heart rate variability

(HRV) as a measure of autonomic nervous system balance, and (vi) blood pressure, with the participants lying in supine position in bed after at least 10min of bedrest and their eyes closed yet awake. Furthermore, capillary blood samples were obtained at regular timepoints for immediate determination of ßHB. Furthermore, acid-base balance, capillary blood gasses, blood glucose and total hemoglobin concentration were measured before hypoxic entry and after 1.5 and 3h in hypoxia. In addition, we collected a venous blood sample before hypoxic entry and after 5h in hypoxia, which was used for determination of plasma glucagon. LLS was evaluated after 4h.

Blood and tissue (prefrontal cortex and skeletal muscle) oxygenation status. Blood oxygen saturation (SpO_2) was measured after 15min and 4h in hypoxia, at 2 Hz using a pulse oximeter (Nellcor PM10N, Medtronic, Minneapolis, USA) with an infrared sensor placed ~2 cm above the left eyebrow. Values were recorded after 10min of bedrest in supine position and data are presented as the average value of the last 30sec. Cerebral and skeletal muscle oxygenation status were assessed by analysis of tissue oxygenation index (TOI) using near infrared spectroscopy (NIRS), also after 15min and 4h in hypoxia. The probes of a NIRO-200 spectrometer (Hamamatsu, Japan) were attached ~2 cm above the right eyebrow for cerebral oxygenation and centrally on the belly of the right *m. vastus lateralis* for skeletal muscle oxygenation. In order to maintain a fixed interoptode distance of 4 cm, crucial to ensure a constant penetration depth of ~2 cm into the muscle/brain tissue, the emitter and detector probes were inserted in a dark-colored rubber spacer. These spacers were attached to the participants using an elastic non-transparent bandage and double-sided adhesive tape to prevent displacement or interference from external light. Before each experimental session, the involved skin was shaved and cleaned to exclude any signal disturbance by hair or impurities. Moreover, the contour lines of the rubber spacer were marked on the skin with a dermatological pen during preparation for the first session, to ensure identical positioning for

every measurement. Participants were asked to preserve and refresh these marks during the washout period in order to maintain this position during the second session. After initial data collection, NIRS data were preprocessed (Matlab R2023a, The Mathworks, Natick, MA) using a fourth-order Butterworth filter with a cut-off frequency of 0.05 Hz [19]. Data were analyzed over 1-min long time chunks.

Cerebral blood flow. Vessel diameter (d) and blood velocity (v) of the internal carotid artery (ICA) and the vertebral artery (VA) through the internal carotid artery (ICA) and the vertebral artery (VA), which constitute the main intracranial blood supply [19], were assessed by a trained and experienced ultrasonographer after 15min and 4h using duplex ultrasonography (Vivid E9, EG Healthcare, New York, USA, with a 9L linear transducer of 2.4 - 10.0 MHz). Measurements were performed in agreement with the technical recommendations as described by Thomas et al. [23]. Artery diameters were measured in the sagittal axes using B-mode imaging, while blood velocity was assessed using pulse-wave mode for later offline analysis. Vessel location and sample volume were determined on an individual basis during the familiarization session, with careful consideration of the diagnostic details of the ICA, and replicated for every measurement. Moreover, the Doppler approach angle was set at 60°, yet angle corrections were applied for tortuous or oblique-angled vessels. Gain and dynamic range were fixed during the familiarization session and unaltered throughout the entire study period. The ICA was assessed at a distance of at least 1.5 cm from the carotid bifurcation in order to guarantee reproducibility and to avoid turbulence. The VA was analyzed between C4 and C5, or alternatively C5 and C6, however the exact location was replicated for each measurement within each participant. Vessel diameters of the ICA and VA (d_{ICA} and d_{VA}, resp.) were measured over 30 sec and 5 consecutive cardiac cycles were included for analysis. Blood velocity (v_{ICA} and v_{VA}, resp.) recordings were collected over 60 sec and analyzed for beat-to-beat TAMEAN using the available ultrasound review software

EchoPAC (GE Healthcare, New York, USA). Blood flow (Q_{ICA} and Q_{VA}, resp.) was calculated as follows: $Q_i = v_i * \left(\frac{d_i}{2}\right)^2 * \pi * 60$ with i being either ICA or VA.,Concurrently, oxygen delivery was calculated as (VA+ICA)*SpO_2 [22].

Ventilatory gas exchange measurements. Indirect calorimetry (Cortex Metalyzer IIIb, Leipzig, Germany) was used during the resting measurement bouts after 15min and 4h in hypoxia to measure breath-by-breath gas exchange data [*i.e.,* minute ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$)]. After main calibration in the morning, the device was kept on for the entire session and recalibrations were executed before every measurement based on actual F_IO_2 values. Data collection started after 10min of bedrest to ensure physiological homeostasis, and data are presented as the average values of the last 5 min.

Blood pressure and heart rate. Resting heart rate and RR intervals (Polar H10, Polar, Kempele, Finland) were measured after 15min and 4h in hypoxia. Data are presented as the average values of the last minute. Heart rate variability (HRV) parameters were assessed using Kubios (Kubios HRV Standard 3.5.0, Kubios Oy, Kuopio, Finland). Blood pressure (Omron M6, Omron healthcare, Kyoto, Japan) was measured before hypoxic entry, as well as after 15min and 4h in hypoxia, on the left arm in sitting position. Systolic (SBP) and diastolic (DBP) blood pressure were used to calculate the mean arterial pressure [MAP = $DBP + \frac{1}{3}(SBP - DBP)$].

Venous blood samples. Before hypoxic entry (baseline) and after 5h in the hypoxic protocol, venous blood samples were obtained from an antecubital vein (Venoject, Terumo, Tokyo, Japan) and collected into vacuum tubes containing EDTA (Becton Dickinson (BD) Vacutainer, Eysins, Switzerland). Tubes were centrifuged (1500 rpm for 10 min at 4°C) and the supernatant was stored at -80°C until later analysis. A commercially available enzyme-

linked immunosorbent assay (ELISA) was performed to determine plasma glucagon levels (DGCG0, R&D Systems, Minneapolis, MN, USA).

Capillary blood samples and analyses. Immediately before and 30min after each supplement, and upon waking up, capillary blood samples were obtained from a hyperaemic earlobe for immediate determination of D-ß-hydroxybutyrate (GlucoMen Areo 2K-meter with ß-ketone sensor strips, A. Menarini Diagnostics, Firenze, Italy). In addition, 70 µl capillary blood was collected from a hyperemic earlobe [treated with topical heating cream (Rado stick, WILL pharma, Wavre, Belgium)] into a capillary tube (safeCLINITUBE, Radiometer Medical ApS, Copenhagen, Denmark) after 5min in seated position (i) before the first supplement, in normoxia, as well as after (ii) 1.5 and (iii) 3h in hypoxia, in resting conditions. After immediate mixing for 10sec, samples were analyzed for acid-base balance, pO_2, and pCO_2 (ABL90 FLEX analyzer, Radiometer Medical ApS, Copenhagen, Denmark).

Statistical analyses and sample size calculation. All statistical analyses were performed in GraphPad Prism version 9.3.1 (GraphPad Software, La Jolla, CA, USA). A nonparametric Wilcoxon matched-pairs signed rank test was used to evaluate the duration of participants' ability to comply to the hypoxic protocol (not normally distributed, D'Agostino Pearson normality test) and for LLS after 4h (discrete variable). Blood β-HB concentrations were analyzed using a mixed-effects model, as early termination of the experimental protocol caused 'missing data'. A two-way repeated measures analysis of variance (ANOVA) was used to evaluate differences between KE and CON and over time for measurements that were performed at multiple timepoints. Sphericity was evaluated using a Mauchly's test, and a Geisser-Greenhouse correction was applied when the criterium of homoscedasticity was not met. When a significant interaction effect was identified, post-hoc analyses were performed using Šidák's correction. When applicable, reported p-values refer to these post-hoc analyses

and otherwise, p-values for main effects were included. All data are presented as mean ± SD unless otherwise stated and statistical significance was defined as $p < 0.05$. The effect size of an earlier study from our laboratory reporting increased blood saturation upon KE during exercise in gradually increasing hypoxia was used for the a-priori sample size calculation [19]. This indicated that a sample size of 10 per condition was required to establish a difference between both conditions for blood oxygen saturation (effect size dz: 0.52; α error: 0.05; power: 0.80; two-sided; ANOVA repeated measures, within-between interaction).

RESULTS

Characteristics of participants.

Sixteen healthy, male participants were initially enrolled in the study with two drop-outs due to Covid-19 infection. Hence, final data analyses reported in the present paper were conducted on fourteen participants (Table 1).

KE induced ketosis and reduced AMS symptoms

The primary outcome of the study was the tolerated time in the hypoxic protocol (*e.g.,* time until severe AMS developed, Fig. 2a,b). From the fourteen participants (AMS_{all}), five participants (36%, AMS_{low}) completed the entire protocol in both conditions. From the other nine participants (AMS_{high}), two completed the protocol in KE only, while seven were unable to complete the protocol in either condition but tolerated the protocol equally long or longer in KE *vs.* CON (Fig. 2c). Hence, upon KE, the tolerated duration increased by 32% when considering all participants [n = 14,mean: ~20h in KE *vs.* 15h in CON, median (IQR): 26h (8-29h) in KE *vs.* 10h (6-29h) in CON, p = 0.008] and doubled when considering only AMS_{high} [n=9, mean: ~15h in KE *vs.* 7.5h in CON, median (IQR): 8h (6-10h) in KE *vs.* 9h (7-26h) in CON, p = 0.008]. After 4h in hypoxia, AMS symptoms were already evident in AMS_{high} and despite no significant difference in AMS_{all}, symptoms were more pronounced in CON *vs.* KE within the AMS_{high} subgroup [n=9, mean: 2 in KE *vs.* 4 in CON, median (IQR): 2 (1-4) in KE *vs.* 4 (3-5) in CON, Fig. 2d, p = 0.047] however not in AMS_{low} [n=5, mean: 2 in KE vs. 4 in CON, median (IQR): 2 (2-4) in KE vs. 0 (0-2) in CON, Fig. 2e, p = 0.088]. Also the score for headache specifically was tempered after 4h upon KE in AMS_{high} [n=9, mean: 0 in KE vs. 1 in CON, median (IQR): 0 (0-1) in KE vs. 1 (1-1.5) in CON, p = 0.021] however not in AMS_{low} [n=5, mean: 1 in KE vs. 0 in CON, median (IQR): 1 (0.5-1) in KE vs. 0 (0-1) in CON, p = 0.178]. Upon early dropout, all participants showed a headache score ≥ 1 [n=7, median (IQR):

296 3 (2-3), range: 1-3 in KE *vs.* n=9, median (IQR): 3 (2-3), range: 1-3 in CON]. Throughout the
297 diurnal parts of the protocol, KE ingestion induced a stable ketosis (*e.g.,* blood [ßHB] of ~3
298 mM, $p < 0.001$ except upon waking up, $p = 0.993$), whereas blood [ßHB] remained low in
299 CON ($p > 0.999$, Fig. 3). Moreover, plasma glucagon levels increased by ~25% in both
300 groups after the first 5h in the hypoxic protocol (Baseline: 76.3 ± 1.4 pg/mL *vs.* 5h: 94.5 ± 0.1
301 pg/mL, $p < 0.001$).

KE mitigated hypoxemia and reduced cerebral blood flow and oxygen delivery

302 Participants' SpO_2 was comparable between KE and CON after 15min of hypoxic exposure (p
303 $= 0.581$). At the 4h timepoint, SpO_2 had further dropped in CON by ~5% but remained stable
304 in KE, indicating that KE attenuated the progressive increase in hypoxemia ($p = 0.017$, Fig.
305 4a). Interestingly, cerebral tissue oxygenation was ~4% higher in KE *vs.* CON after 4h ($p =$
306 0.049, Fig. 4b), while blood flow through the ICA and VA was consistently ~20-25% lower in
307 KE ($p = 0.008$ and $p = 0.014$, Fig. 4c-d, resp.). The KE-induced decrease in cerebral blood
308 flow resulted from a drop in blood velocity ($p < 0.014$) and a trend towards lower arterial
309 diameters ($p < 0.091$, Table S1). This KE-induced drop in cerebral blood flow caused total
310 cerebral oxygen delivery to be consistently lower in KE *vs.* CON ($p = 0.004$, Fig. 4e). Similar
311 to the cerebral oxygen status, skeletal muscle oxygenation index also increased from 15min to
312 4h in KE, while remaining stable in CON ($p < 0.001$, Fig 4f).

KE shifted the autonomic nervous system towards increased sympathetic activity

313 KE consistently increased resting heart rate ($p = 0.036$, Fig. 5a), while reducing the
314 percentage of adjacent NN intervals that differ by more than 50 ms (pNN50, $p = 0.018$, Fig.
315 5b), the root mean square of successive differences (RMSSD, $p = 0.004$, Fig. 5c), as well as
316 power of the high-frequency band (HF, $p = 0.027$, Fig. 5d) which are all indicative of a shift

317 towards increased sympathetic activity. This shift occurred without alterations in blood
318 pressure ($p = 0.48$, Table S2).

KE increased the hypoxic ventilatory response and altered acid-base balance

319 The improved hypoxemia may not only result from a reduction in tissue oxygen demand [24],
320 but can also result from an increased ventilatory response [19]. In this perspective, KE
321 consistently elevated ventilation ($\dot{V}_E$, $p = 0.014$), as well as increased oxygen uptake rate
322 ($\dot{V}O_2$, $p = 0.022$) after 15min in hypoxia (Table 2). Also, KE decreased end tidal CO_2
323 ($PetCO_2$, $p < 0.001$, Table 2). Yet between 15min and 4h, $\dot{V}O_2$ increased in CON but not in
324 KE, resulting in similar values at 4h ($p = 0.191$). Acid-base balance and capillary blood gasses
325 are shown in Table 3. Compared to CON, KE consistently decreased capillary pH, $[HCO_3^-]$
326 and pCO_2 ($p < 0.001$ for all), while increasing p50 ($p < 0.001$). Conversely, capillary pO_2
327 decreased to a similar extent in both conditions after 1.5h in hypoxia but was higher in KE *vs.*
328 CON after 3h ($p < 0.001$). KE consistently lowered capillary glucose concentration by ~0.8 to
329 1.1 mM compared to CON ($p < 0.001$), while total hemoglobin concentration similarly
330 increased in both groups from baseline to 1.5h in hypoxia ($p < 0.001$). After 3h in hypoxia,
331 total hemoglobin concentrations had returned to baseline levels in both KE and CON ($p =$
332 0.101).

333 **Blinding of supplementation**

334 After the final experimental session, participants were asked to identify which supplement
335 they received during each session. They were also asked how confident they were in their
336 choice by means of a continuous score ranging from 0% (no idea at all) to 100% (completely
337 certain). Out of 14 participants, 8 were 0%-50% (6 out of 8 guessed right) and 6 participants
338 were 80-90% sure (and guessed right). However, conversations with the participants revealed
339 that these decisions were based on how they felt during their session (expecting to feel better

in KE) rather than on the taste of the supplements. Indeed, the participants that indicated to be 80-90% sure were participants who stayed longer in their ketone session and the group of participants with low AMS incidence all indicated to be 0-50% sure.

DISCUSSION

The present work sought to investigate whether increasing blood KB can alleviate hypoxemia in human participants and thereby alleviate the development/severity of AMS. For this purpose, participants underwent a 29h protocol twice at a simulated altitude of 4,000-4,500m involving intermittent exercise bouts, while receiving either KE or CON. Our results indicate that KE increased tolerated duration of simulated high-altitude exposure and reduced AMS symptoms. This was accompanied by an attenuation of the progressive decrement in arterial oxygen saturation during hypoxic exposure, and an increase in both cerebral and muscular oxygenation status. These observations were associated with a KE-induced increase in both ventilation and pO_2, together with a reduction in cerebral oxygen delivery potentially indicating that KE could reduce cerebral oxygen consumption. Furthermore, KE consistently reduced cerebral blood flow and likely shifted the autonomic nervous system towards increased sympathetic activity, based on decreases in HRV indices pNN50, RMSSD, and HF, which may all together underly the observed attenuation of AMS [10].

A high degree of inter-individual variability in AMS susceptibility is well established. In this regard, the normobaric hypoxic protocol elicited AMS in 64% (9 out of 14) of the individuals in the CON condition, resulting in an average tolerated duration of ~15h. This approximates the expected incidence at terrestrial altitude given earlier studies reporting an incidence rate of 53% in mountaineers at 4,243m [3] and 4,559m altitude [25]. Interestingly, KE ingestion completely negated the development of AMS in 2 individuals, as well as increased protocol duration by 32% on average and by 99% in AMS-developing participants.

The primary trigger for AMS is a hypoxia-induced drop in arterial oxygen saturation. This is clearly exemplified by the nearly immediate recovery from AMS upon administration of oxygen or hyperbaria [26], and the direct relationship between the extent of hypoxemia and

AMS incidence and severity [6]. In our study, SpO_2 values dropped to ~82% after 15min of hypoxic exposure in both conditions, which is in perfect agreement with the expected SpO_2 at the given altitude [27]. By the 4h timepoint, SpO_2 values had dropped by an additional 5% in CON, which most likely reflects an inability to cope with the hypoxic stress. This could partly be attributed to an increased accumulation of interstitial fluid in the lungs, causing an impaired pulmonary gas exchange [10, 28]. Moreover, both the increased energetic demand during the maximal and submaximal exercise bout could establish increased tissue oxygen demand and therefore incomplete recovery of blood oxygenation [29, 30]. Remarkably, this final drop in oxygen saturation was fully negated by KE supplementation. Alternatively, this observed decline in saturation could be attributed to the nadir only occurring after this 15min timepoint [31]. In this regard, saturation would not have further dropped after this nadir in CON but rather stayed stable whereas SpO_2 may have partially recovered at the 4h timepoint in KE.

This KE-induced attenuation of oxygen desaturation is in line with the results from an earlier study by our research group [19]. In this study, KE negated the drop in oxygen saturation during the final ~30min of a 3h submaximal cycling bout wherein simulated altitude gradually increased from 1,000m to 3,000m. Interestingly, both in our earlier work as well as in the current study, SpO_2 values were increased by KE relative to CON only after a few hours of hypoxic/ketone exposure in combination with exercise (*e.g.,* ~2.5-4h), but not during the earlier timepoints (*e.g.,* ~15min-2h). The extent of altitude/hypoxemia was more pronounced after 15min in the current study than after 2h in the earlier study. This suggests that the ability of KE to raise blood oxygen saturation is independent of the extent of altitude/hypoxemia, but rather requires a given period of (hypoxic) time or exercise load. This time-dependent effect may for instance be linked to the levels of glucagon as earlier data showed that simultaneous administration of glucagon and ßHB, but not glucagon nor ßHB alone, doubled hypoxic

survival time in mice [16]. In this regard, plasma glucagon levels increased by 25% after 5h in hypoxia. Another explanation for the delayed improvement in oxygenation with KE might be related to the earlier observation that KB only significantly contribute to skeletal muscle energy metabolism upon prolonged (i.e., minimal a few hours) KB exposure [32, 33].

Cerebral blood flow is highly sensitive to blood pCO_2. As such, the consistent KE-induced increase in end tidal pCO_2 and drop in capillary pCO_2 most likely resulted in cerebral vasoconstriction and a decreased cerebral blood flow. This, however, was not reflected in previous research [34, 35] in normoxic conditions. In combination with similar total hemoglobin concentrations in KE and CON, this indicates that cerebral oxygen delivery was consistently lower in KE compared to CON. Nevertheless, cerebral oxygenation was similar between both conditions at the 15min timepoint. This is in line with the suggestion that KE might reduce oxygen consumption by the brain, potentially indicating improved mitochondrial oxygen efficiency. However this did not coincide with changes in SpO_2 or pO_2, which were similar between KE and CON at 15min. This was probably explained by the consistent ketosis-induced hyperventilation which, in line with previous research [19, 36], concomitantly provoked a higher $\dot{V}O_2$ in KE compared to CON and thus fully compensated the lowered cerebral oxygen demand. The observed, delayed attenuation of arterial oxygen desaturation with KE can either result from increased arterial pO2 or a left-shift of the oxyhemoglobin dissociation curve. Our results indicate that the former mechanism was at play given that the KE-induced acidosis caused a right-shift of the curve (increased p50), while capillary pO2 values were higher in KE *vs.* CON after 3h. It should be highlighted that pO2 values were obtained using a capillary sampling method, which might underestimate actual arterial pO2. Nevertheless, these data are in line with earlier observations showing an increase in arterial, capillary and hippocampal oxygenation [19, 37, 38] upon endogenous and exogenous ketosis. In turn, an increased pO2 can either result from a higher oxygen uptake

416 through increased ventilation or from a decreased cellular oxygen consumption. After
417 prolonged hypoxic exposure, a gradually augmented hypoxic ventilatory response increased
418 $\dot{V}O_2$ in CON, which likely promoted the observed drop in oxygen saturation from 15min to
419 4h. Despite a slight additional increase in hyperventilation in KE, oxygen consumption did
420 not further increase and SpO_2 values remained stable. Interestingly, KE intake increased
421 cerebral oxygenation after 4h, even in combination with the before mentioned lower cerebral
422 oxygen delivery, suggesting a potential further alteration in mitochondrial oxygen efficiency
423 after prolonged exposure. Such effect may have also been present in other tissues given the
424 observed KE-induced increase in oxygenation status of the *m. vastus lateralis*, and supports
425 earlier *ex-vivo* data indicating that KB can increase mechanical work to oxygen ratio [24].

426 Hypoxemia is considered to evoke AMS via multiple mechanisms, with recent evidence
427 indicating that the specific pathophysiological mechanism depends on the time course by
428 which AMS develops [10]. In this perspective, AMS development during the first 29h is
429 mostly caused by an increase in (i) relative parasympathetic activity, and (ii) cerebral blood
430 flow. Both parasympathetic contribution, as evidenced by resting heart rate, and the HRV
431 indices pNN50, RMSSD, and HF power, as well as blood flow in both the ICA and VA, the
432 main arteries for intracranial blood supply, were markedly reduced upon KE. This suggests
433 that KE may have lowered AMS symptoms via both mechanisms. An increase in sympathetic
434 drive has also been reported in some, but not all, earlier studies looking at the acute effect of
435 ketosis on sympathetic activity in normoxia [39–41], and may amongst others result from a
436 KE-induced suppression of atrial natriuretic peptide [41]. This suggests that the observed
437 increase in sympathetic tone in our study likely directly results from ketosis as the KE-
438 induced attenuation of hypoxemia would rather attenuate sympathetic stimulation. In contrast,
439 cerebral blood flow has consistently been shown to improve during acute ketosis under
440 normoxic conditions both in rats [42], and in healthy and obese adults [34, 35]. This suggests

that the KE-induced reduction in cerebral blood flow observed in our study was not a primary effect of ketosis, but occurred as a secondary effect mediated by the increase in oxygen saturation upon KE.

Collectively, these data indicate that KE ingestion may evolve as a novel, non-pharmacological intervention to improve hypoxemia at high altitude and alleviate AMS. Although a gradual ascent is still the best strategy to prevent AMS [43], the carbonic anhydrase inhibitor acetazolamide (AZ) is currently considered as the preferred drug to reduce AMS [14]. Interestingly, the mechanism by which AZ increases hypoxic tolerance shows some analogy with the observed physiological effects of KE. Similar to AZ-induced inhibition of carbonic anhydrase (CA) [44], βHB has been shown to exert an inhibitory effect on human CA activity [45] and therefore might provoke a similar physiological response. Administration of AZ causes a bicarbonate diuresis thereby generating a metabolic acidosis which via an increased ventilatory drive results in better arterial oxygenation [13]. Nevertheless, there are also some profound differences given that AZ for instance induces diuresis at high dosages [46], while KE is rather anti-diuretic [41]. It would be inappropriate to compare the effectiveness of AZ with KE given the limited sample size of our study. Nevertheless, the observed increase in arterial oxygen saturation upon KE (~+4%) was at least similar as typically seen following AZ (~+2 to 5%) at comparable altitudes [47, 48].

Despite providing novel insights, we would like to acknowledge a few limitations of the present study. In most cases, AMS manifests within the timeframe of the study (*e.g.,* within 29h following acute altitude exposure). But, in some participants symptom severity peaks at a slightly later timepoint, particularly triggered by subclinical pulmonary edema [10]. As such, it remains to be determined if KE can also prove beneficial for this AMS type. Second, it should be highlighted that our study was performed in normobaric hypoxia. Earlier research clearly showed that the response to normobaric *vs.* hypobaric hypoxia elicits comparable

physiological effects [49, 50], yet also minor differences exist such as slightly higher AMS scores in hypobaric hypoxia [51]. No normoxic baseline values were included for our resting measurements (cerebral blood flow, HRV, respiratory gas exchange, cerebral and tissue oxygenation) due to practical considerations. Along a similar line, resting measurements were not only performed after 15min and 4h in hypoxia, however also after 10h, 24h and 28h. These data were not included as high drop-out rates because of AMS development caused sample sizes to decrease down to n=7, n=5 and n=5, resp. Moreover, estimation of oxygen delivery according to the formula above neglects any changes in blood volume and therefore hemoglobin concentrations. As the latter was only measured at different timepoints, we can only conclude that no considerable differences in hemoglobin concentration occurred between KE and CON, however different timepoints are not to be compared. Notably, only males were recruited for participation given the debated and ambiguous role of the hormonal cycle on for example the hypoxic ventilatory response [52] as well as ventilatory parameters ($\dot{V}E/\dot{V}O_2$ and $PetCO_2$) [53] and menstruation related discomfort potentially influencing AMS scoring. Therefore, it remains to be identified if similar effects would also be observed in females, who have inconsistently been shown to have a potential higher incidence of AMS [2, 54]. Moreover, the abrupt transition from sea level to hypoxia deviates from realistic expedition patterns. Recent research however showed similar effects on SpO_2 in gradually increasing altitudes [19]. AMS development is also inversely related to energy intake [55]. In this perspective, our placebo drink contained less calories compared to the KE drink. But it is unlikely that this may have provoked the observed effects given that all participants received a caloric surplus, and given that lower energy intake is rather a consequence than a cause of AMS [56]. Moreover, employing an isocaloric placebo containing either carbohydrates or fats would considerably alter substrate metabolism and therefore we decided to employ an inert placebo.

In conclusion, our data are the first to indicate that exogenous ketosis attenuates the development of AMS. This is mediated by an improved arterial oxygenation in combination with increased cerebral and muscular oxygenation, lower cerebral blood flow, and increased sympathetic dominance. The increase in cerebral oxygenation occurred along with a reduction in cerebral oxygen supply, thereby suggesting the first *in-vivo* human data to indicate that ketosis could lower cerebral oxygen demand at high altitude.

ADDITIONAL INFORMATION

Author contributions: All experiments were performed within the Exercise Physiology Research Group and the Bakala Academy-Athletic Performance Center at the KU Leuven, Belgium. Conception and design of the study: MS, TD and CP. Data collection and/or data analyses: MS, DT, WL, RR, TD and CP. Interpretation of the data and manuscript drafting: MS and CP. All authors critically revised the manuscript and approved the final version of the manuscript.

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Supplemental information

Supplemental Table S1:

URL: https://figshare.com/articles/dataset/Table_S1/26496193?file=48177799

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Supplemental Table S2

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FIGURES

Figure 1. Schematic representation of the experimental sessions. In a double-blind, randomized, crossover design, 14 participants underwent a 29h normobaric hypoxic protocol (26h at 4,000m, followed by 3h at 4,500m) that was explicitly designed to elicit acute mountain sickness (AMS). Participants were instructed to comply to the protocol, but it was prematurely terminated if they developed severe AMS (Lake Louise score ≥ 10 out of 15). Throughout the entire protocol, participants received either ketone ester (KE) or placebo (CON) supplements at regular timepoints through portions of either 25g (big arrow) or 12.5g (small arrow). In each session, 2 maximal (10min warming up + ramp protocol), 4 submaximal (30min) and 1 combined exercise bout (40min + ramp protocol) were completed. Moreover, after 15min and 4h in hypoxia, the following resting measurements were performed in order to characterize the physiological response: blood oxygen saturation, cerebral and muscular oxygenation status, cerebral blood flow, respiratory gas exchange, heart rate, heart rate variability, and blood pressure. In addition, before hypoxic entry, as well as after 1.5 and 3h in hypoxia, a capillary blood sample was collected for determination of acid-base balance, pO_2 and pCO_2.

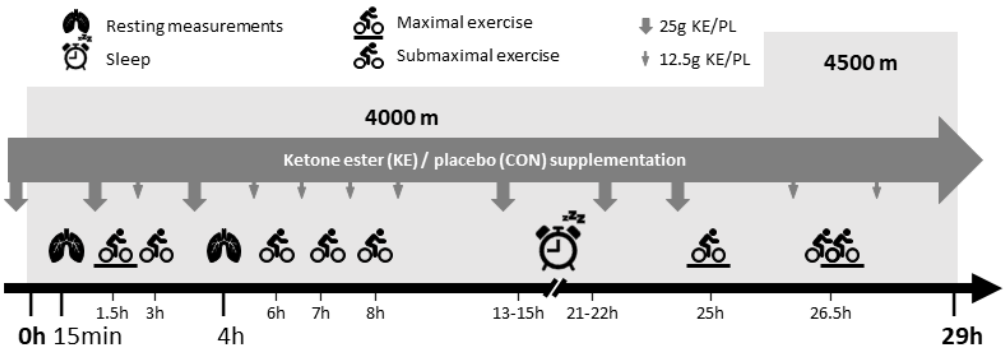
Figure 2. (a) Individual data points showing participants' tolerated time in hypoxia in response to ingestion of ketone ester (KE, red) *vs.* placebo (CON, black) during a 29h normobaric hypoxic protocol. (n=5) indicates the 5 participants that completed both experimental sessions. (b) Protocol duration plot of participants and drop-out due to severe AMS development. (c) Individual differences together with mean ± 95% confidence interval for tolerated time in hypoxia between the KE and CON session, with a distinction between participants that developed severe AMS in at least one of both sessions (AMS_{high}, n=9, full circles) and participants that never developed severe AMS (AMS_{low}, n=5, open circles). Data are shown for n=14. (d-e) Median (bar plots) ± IQR (whiskers) as well as individual Lake Louise Scores (LLS) are shown after 4h in hypoxia. Both sessions of an individual participant are connected. Data are shown for AMS_{high} (d, n = 9) as well as AMS_{low} (e, n = 5). For the single participant that developed severe AMS before the 4h timepoint, we included the value that was noted down upon hypoxic leave. However, potential exclusion of this participant did not alter the statistical difference between CON and KE. (f) Median (bar plots) ± IQR (whiskers), as well as individual Lake Louise Scores (LLS) over all timepoints. Early drop-out due to severe AMS caused missing datapoints starting at the 9.5h timepoint. *, $p < 0.05$ between KE and CON.

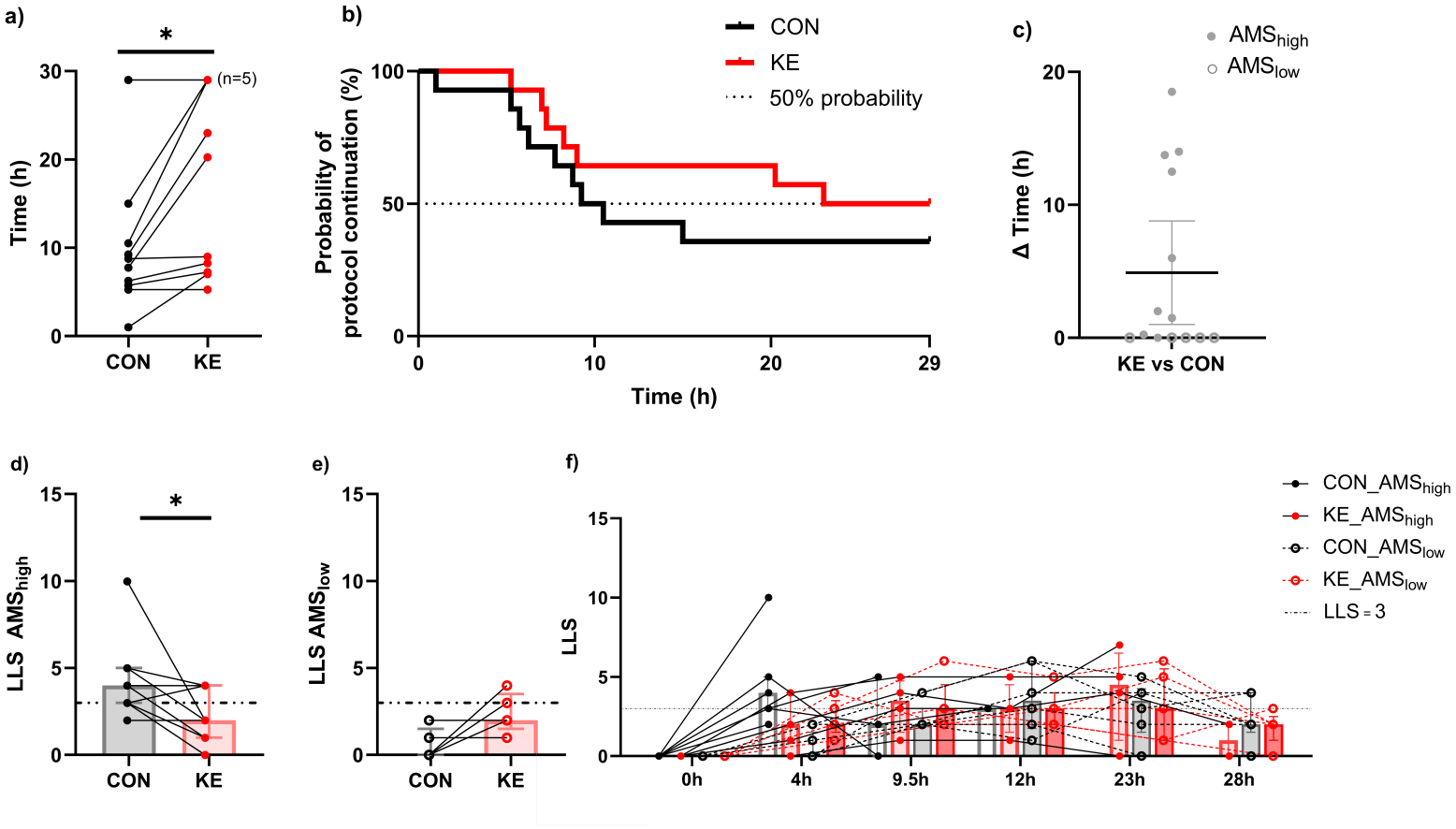
Figure 3. Mean ± SD data representing blood D-β-hydroxybutyrate concentration ([βHB]) in response to ingestion of ketone ester (KE, red) *vs.* placebo (CON, black) during a 29h normobaric hypoxic protocol. Grey area indicates the time during which the participants were sleeping. Data are means of all participants at the start (n=14) however sample size decreases upon early protocol termination. *, $p < 0.05$ between KE and CON; §, $p < 0.05$ *vs.* baseline for indicated condition.

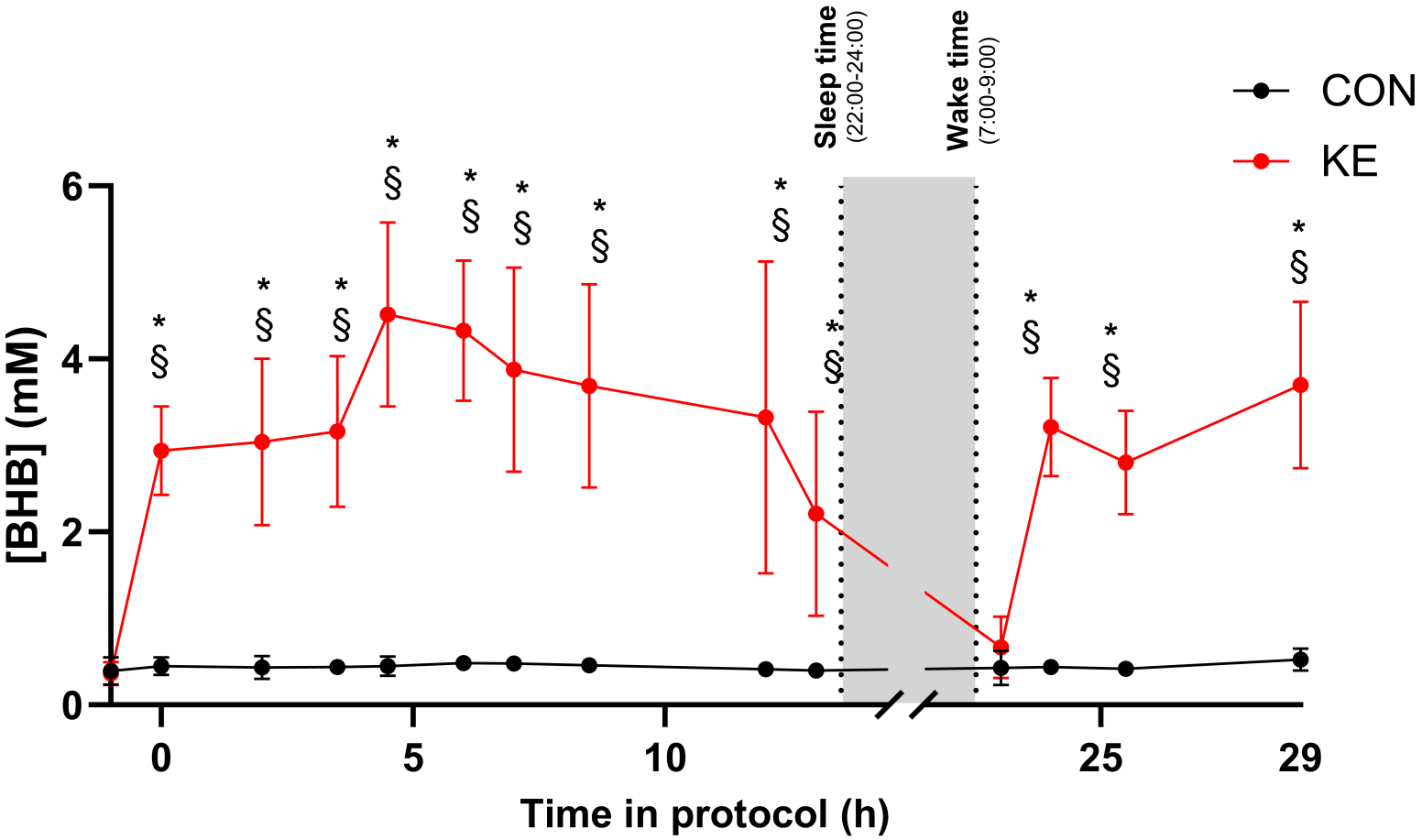
721 **Figure 4.** Mean (bar plots) ± SD (whiskers), as well as individual values for (a) arterial
722 oxygen saturation, (b) cerebral (prefrontal cortex) tissue oxygenation index (TOI), (c) blood
723 flow in the internal carotid artery (ICA), (d) blood flow in the vertebral artery (VA), and (e)
724 cerebral oxygen delivery via the ICA and VA, and (f) muscular TOI. Measurements were
725 performed after 15min and 4h of a 29h hypoxic protocol involving intermittent ketone ester
726 (KE, red) or placebo (CON, black) ingestion. Data are shown for n=13 and individual's
727 sessions are connected. Participants that completed both experimental sessions without
728 development of severe AMS were attributed an empty circle, and participants that developed
729 severe AMS in at least one of both sessions, leading to premature termination of the protocol,
730 are depicted by full circles. No data were included for the participant that developed severe
731 AMS after 1h in CON. *, $p < 0.05$ between KE and CON; §, $p < 0.05$ *vs.* 15min for indicated
732 condition.
733

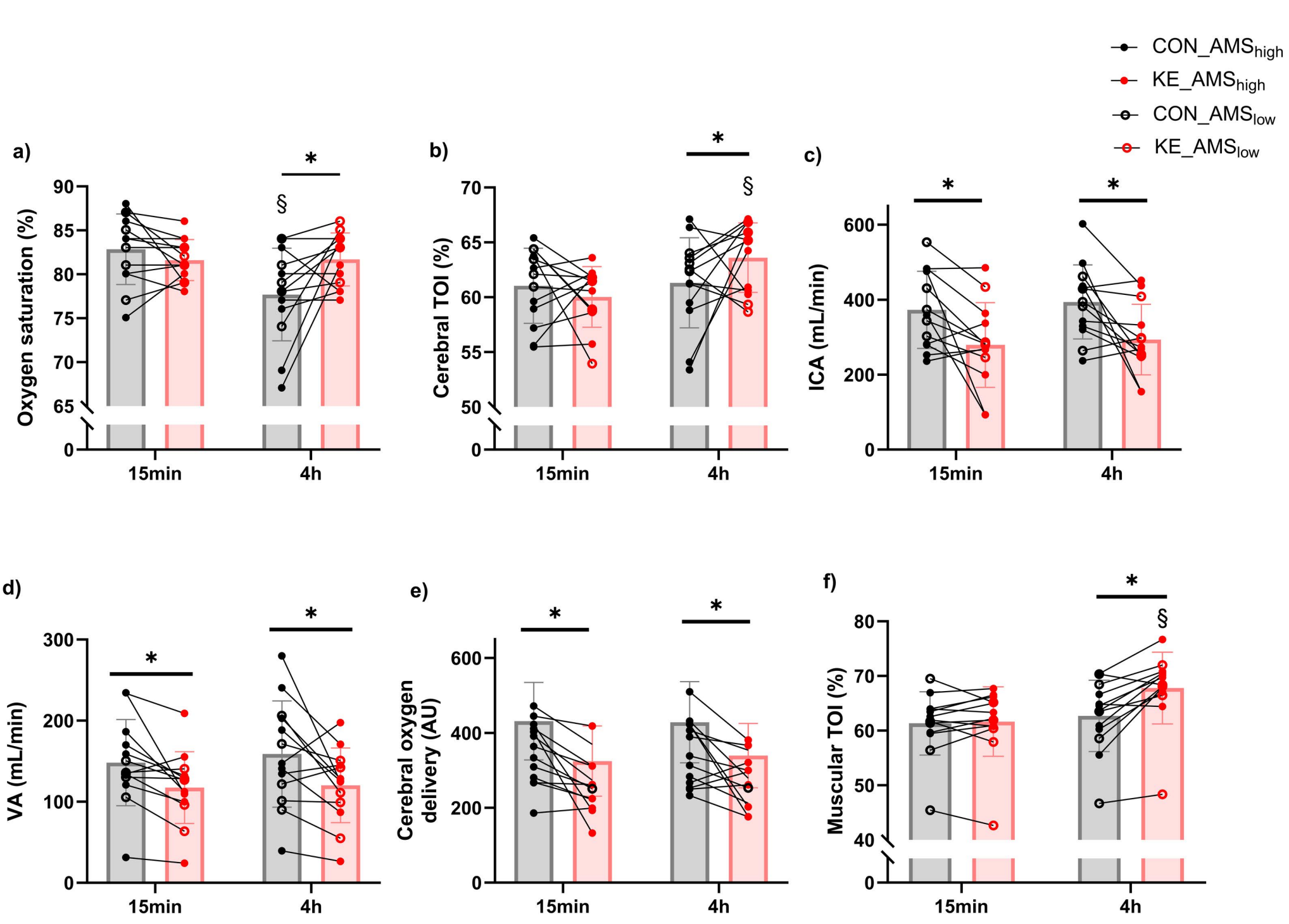
734 **Figure 5.** Mean (bar plots) ± SD (whiskers), as well as individual values for (a) heart rate, (b)
735 the percentage of adjacent NN intervals that differ by more than 50 ms (pNN50), (c) the root
736 mean square of successive differences (RMSSD), and (d) the power of the high-frequency
737 band (HF). Measurements were performed after 15min and 4h of a 29h hypoxic protocol
738 involving intermittent ketone ester (KE, red) or placebo (CON, black) ingestion. Data are
739 shown for n=13 and individual's sessions are connected. Participants that completed both
740 experimental sessions without development of severe AMS were attributed an empty circle,
741 and participants that developed severe AMS in at least one of both sessions, leading to
742 premature termination of the protocol, are depicted by full circles. No data were included for
743 the participant that developed severe AMS after 1h in CON. *, $p < 0.05$ between KE and
744 CON; #, $p < 0.05$ *vs.* 15min for both conditions.
745

746


Resting measurements
Sleep
Maximal exercise
Submaximal exercise
25g KE/PL
12.5g KE/PL
4500 m
4000 m
Ketone ester (KE) / placebo (CON) supplementation
0h
15min
1.5h
3h
4h
6h
7h
8h
13-15h
21-22h
25h
26.5h
29h


a)
*
(n=5)
Time (h)
30
20
10
0
CON
KE
b)
CON
KE
50% probability
Probability of protocol continuation (%)
100
50
0
0
10
20
29
Time (h)
c)
AMS$_{high}$
AMS$_{low}$
Δ Time (h)
20
10
0
KE vs CON
d)
*
LLS AMS$_{high}$
15
10
5
0
CON
KE
e)
LLS AMS$_{low}$
15
10
5
0
CON
KE
f)
LLS
15
10
5
0
0h
4h
9.5h
12h
23h
28h
CON_AMS$_{high}$
KE_AMS$_{high}$
CON_AMS$_{low}$
KE_AMS$_{low}$
LLS = 3


Sleep time
(22:00-24:00)
Wake time
(7:00-9:00)
CON
KE
[BHB] (mM)
6
4
2
0
0
5
10
25
29
Time in protocol (h)


CON_AMS$_{high}$
KE_AMS$_{high}$
CON_AMS$_{low}$
KE_AMS$_{low}$
a)
Oxygen saturation (%)
90
85
80
75
70
65
0
15min
4h
*
*
§
b)
Cerebral TOI (%)
70
65
60
55
50
0
15min
4h
*
*
§
c)
ICA (mL/min)
600
400
200
0
15min
4h
*
*
d)
VA (mL/min)
300
200
100
0
15min
4h
*
*
e)
Cerebral oxygen delivery (AU)
600
400
200
0
15min
4h
*
*
f)
Muscular TOI (%)
80
70
60
50
40
0
15min
4h
*
*
§

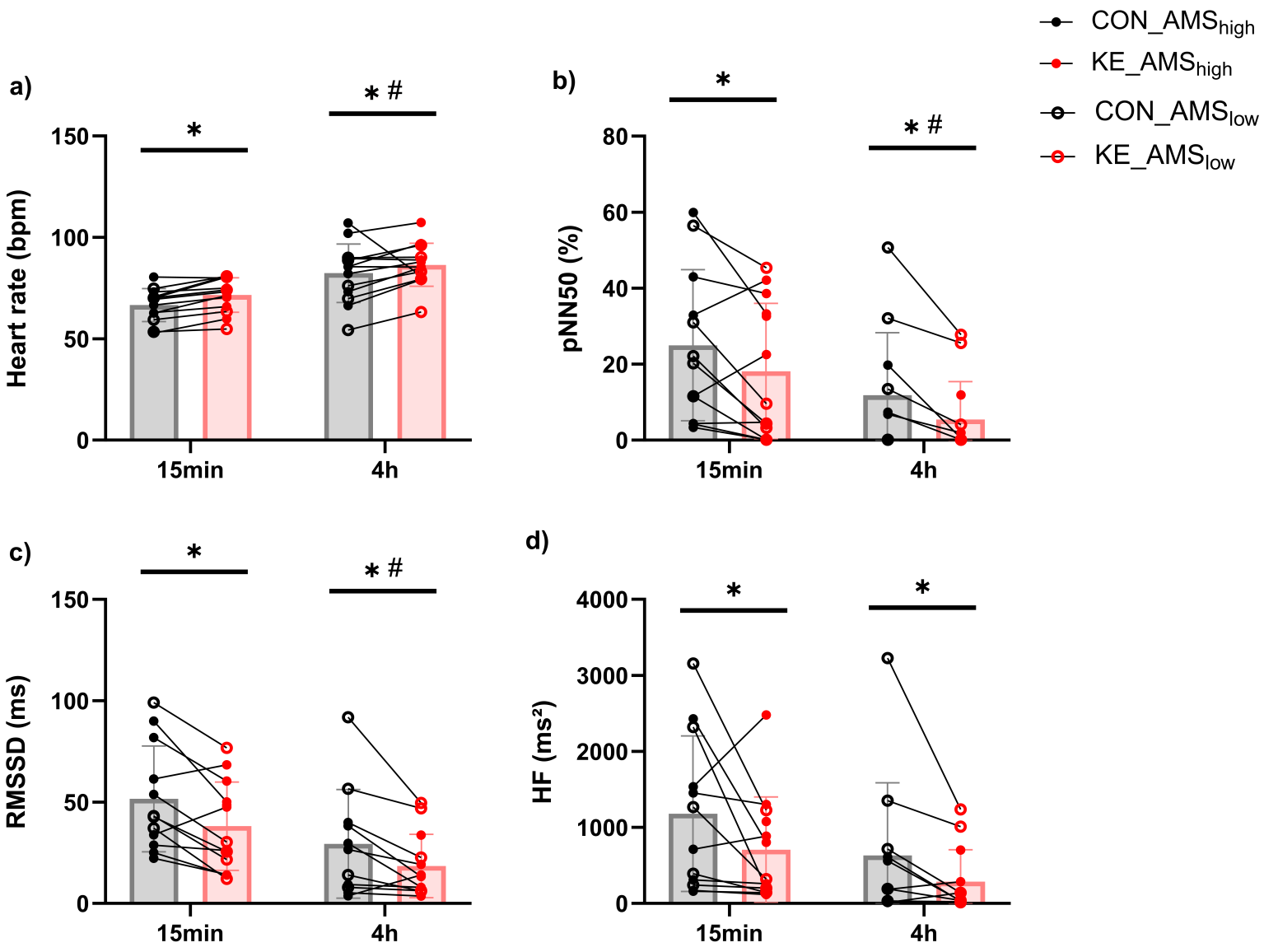

a)
b)
c)
d)
CON_AMS$_{high}$
KE_AMS$_{high}$
CON_AMS$_{low}$
KE_AMS$_{low}$
Heart rate (bpm)
pNN50 (%)
RMSSD (ms)
HF (ms^2)
15min
4h
*
* #

Table 1. Participants' baseline characteristics.

Age (yr)	26 ± 5
Height (m)	1.84 ± 0.05
Body mass (kg)	73.9 ± 4.0
$\dot{V}O_2$max (mL.kg^{-1}.min^{-1})	52.0 ± 6.7

Data are means ± SD and represent baseline characteristics of the 14 included participants.

Table 2. Effect of ketone ester (KE) vs placebo (CON) ingestion on respiratory gas exchange parameters.

	CON	KE
$\dot{V}_E$ (L.min^{-1})		
15min	11.9 ± 1.6	12.9 ± 1.6 *
4h	13.4 ± 1.8 #	14.7 ± 1.2 #*
$\dot{V}O_2$ (L.min^{-1})		
15min	0.34 ± 0.04	0.37 ± 0.06 *
4h	0.36 ± 0.06 §	0.35 ± 0.04
$\dot{V}CO_2$ (L.min^{-1})		
15min	0.35 ± 0.04	0.37 ± 0.05
4h	0.31 ± 0.05 #	0.29 ± 0.03 #
$PetCO_2$ (mmHg)		
15min	35.9 ± 1.9	34.5 ± 2.1 *
4h	28.1 ± 1.1 #	25.4 ± 1.6 #*

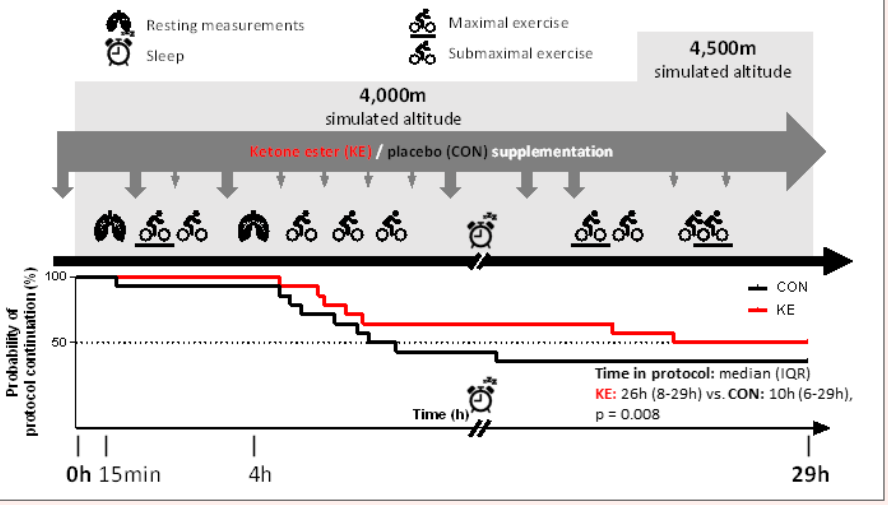
Respiration was evaluated after 15min and 4h of a 29h hypoxic protocol in participants receiving either ketone ester (KE) or placebo (CON) drinks. $\dot{V}E$, ventilation; $\dot{V}O2$, oxygen consumption; $\dot{V}CO2$, carbon dioxide production; $PetCO_2$, end tidal partial CO_2 pressure. Data are means ± SD (n = 13). No data were included for the participant that developed severe AMS after 1h in CON. *, $p < 0.05$ *vs.* CON; #, $p < 0.05$ vs. 15min in both conditions; §, $p < 0.05$ *vs.* 15min for indicated condition.

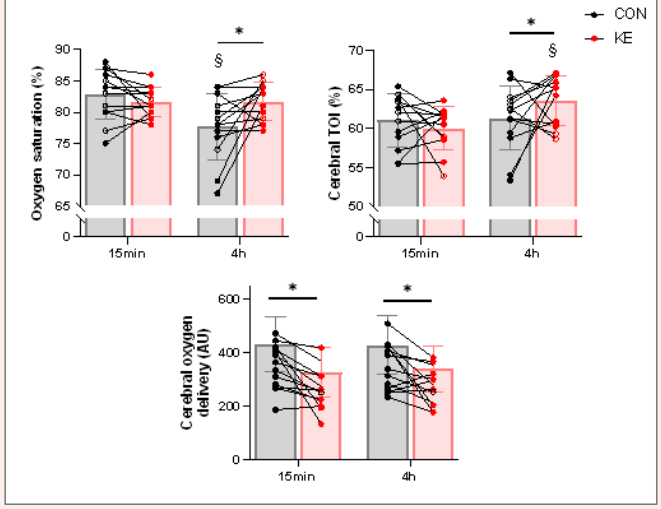
Table 3. Effect of ketone ester (KE) vs placebo (CON) ingestion on capillary blood gasses, acid-base balance, blood glucose and total hemoglobin concentration.

	CON	KE
pH		
Baseline	7.414 ± 0.019	7.421 ± 0.012
1.5h	7.432 ± 0.017 §	7.395 ± 0.022 *§
3h	7.450 ± 0.018 §	7.392 ± 0.025 *§
$[HCO_3^-]$ (mM)		
Baseline	25.7 ± 1.1	26.0 ± 0.8
1.5h	25.8 ± 0.9	22.7 ± 1.0 *§
3h	25.3 ± 1.1	21.0 ± 1.5 *§
pCO_2 (mmHg)		
Baseline	41.3 ± 1.8	40.7 ± 1.3
1.5h	40.0 ± 2.7	37.6 ± 1.9 *§
3h	36.3 ± 1.8 #	33.2 ± 1.8 *#
p50 (mmHg)		
Baseline	25.13 ± 1.57	24.74 ± 1.30
1.5h	25.31 ± 1.00	26.32 ± 0.97 *§
3h	24.68 ± 0.99	26.34 ± 0.99 *§
pO_2 (mmHg)		
Baseline	84.3 ± 8.3	84.6 ± 6.5
1.5h	42.0 ± 2.7 #	43.9 ± 3.0 #
3h	40.9 ± 3.0 #	46.3 ± 3.2 *#
Glucose (mM)		
Baseline	4.96 ± 0.36	5.03 ± 0.28
1.5h	5.51 ± 0.55 §	4.68 ± 0.61 *
3h	5.95 ± 0.8 §	4.87 ± 0.59 *
Total Hb (g/dL)		
Baseline	15.8 ± 1.5	15.8 ± 1.2
1.5h	16.7 ± 1.6 #	16.7 ± 1.6 #
3h	15.1 ± 1.1	15.6 ± 1.3

Capillary blood gasses and acid-base balance were evaluated in capillary blood samples. Samples were obtained before hypoxic entry (baseline), and after 1.5 and 3h of a 29h hypoxic protocol involving intermittent ketone ester (KE) or placebo (CON) ingestion. Data are means ± SD (n = 13). No data were included for the participant that developed severe AMS after 1h in CON. *, $p < 0.05$ *vs.* CON; #, $p < 0.05$ *vs.* baseline in both conditions; §, $p < 0.05$ *vs.* baseline for indicated condition.

Exogenous ketosis attenuates acute mountain sickness and mitigates normobaric high-altitude hypoxemia.





Ketone ester intake attenuated the development of acute mountain sickness at a simulated altitude of 4,000-4,500m.
This likely resulted from a mitigation of arterial and cerebral hypoxemia, reduced cerebral oxygen delivery and increased sympathetic drive.