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A Single Night in Hypoxia Either with or without Ketone Ester Ingestion Reduces Sleep Quality without Impacting Next-Day Exercise Performance

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ABSTRACT

STALMANS, M., D. TOMINEC, R. ROBBERECHTS, W. LAURIKS, M. RAMAEKERS, T. DEBEVEC, and C. POFFÉ. 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.*, Vol. 57, No. 4, pp. 807–819, 2025. **Background:** Sleeping at altitude is highly common in athletes as an integral part of altitude training camps or sport competitions. However, concerns have been raised because of expected negative effects on sleep quality, thereby potentially hampering exercise recovery and next-day exercise performance. We recently showed that ketone ester (KE) ingestion beneficially impacted sleep after strenuous, late evening exercise in normoxia, and alleviated hypoxemia. Therefore, we hypothesized that KE ingestion may be an effective strategy to attenuate hypox(em)ia-induced sleep dysregulations. **Methods:** Eleven healthy male participants completed three experimental sessions including normoxic training and subsequent sleep in normoxia or at a simulated altitude of 3000 m while receiving either KE or placebo postexercise and presleep. Sleep was evaluated using polysomnography, whereas next-day exercise performance was assessed through a 30-min all-out time trial ($TT_{30'}$). Physiological measurements included oxygen status, heart rate variability, ventilatory parameters, blood acid–base balance, and capillary blood gases. **Results:** Hypoxia caused a ~3% drop in sleep efficiency, established through a doubled wakefulness after sleep onset and a ~22% reduction in slow wave sleep. KE ingestion alleviated the gradual drop in SpO_2 throughout the first part of the night, but did not alter hypoxia-induced sleep dysregulations. Neither KE nor nocturnal hypoxia affected $TT_{30'}$ performance, but nocturnal hypoxia hampered heart rate recovery after $TT_{30'}$. **Conclusions:** We observed that sleeping at a 3000 m altitude impairs sleep efficiency. Although this hypoxia-induced sleep disruption was too subtle to limit exercise performance, we for the first time indicate that sleeping at altitude might impair next-day exercise recovery. KE alleviated nocturnal hypoxemia only when SpO_2 values dropped below ~85%, but this did not translate into improved sleep or next-day exercise performance. **Key Words:** EXERCISE PERFORMANCE, HYPOXIA, KETONE, OXYGEN SATURATION, RECOVERY, SLEEP

Sleeping at altitude is highly common in athletes as an integral part of altitude training camps (1). However, athletes are also often enforced to sleep at altitude during major sport competitions such as grand tours in cycling, Olympic Games, multiday ultrarunning races, or mountaineering expeditions (2). During altitude training camps, nocturnal hypoxic exposure is considered essential for inducing favorable physiological adaptations in the longer term such as increased hemoglobin mass and maximal oxygen consumption rates, which may ultimately improve endurance exercise performance (3). Conversely, sleeping at altitude evokes serious concerns in athletes—especially during competitions—because of the expected negative effects of hypoxia on sleep quality (4), which may in turn hamper exercise recovery and next-day exercise performance (5).

In this respect, studies generally observed that a single night at (simulated) altitudes above 2000 m impairs sleep quality

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(4,6–13). This is evidenced by decreases in either rapid eye movement (REM) or slow wave sleep (SWS), or an increase in wakefulness during the night. Methodological heterogeneity and contradictory findings, however, hinder unequivocal conclusions and a clear rationale. In general, REM sleep is unaffected at altitudes up to 3800 m (4,7,9,10,14–16) but decreases up to 40% at altitudes starting from 4000 m (6,8,12,13). Data on SWS are much more equivocal with some studies reporting no changes at altitudes up to 3800 m (10,14,15), whereas other studies reported decreases from ~20% to 45% at altitudes between 2000 and 4300 m (4,8,9,12,16). This is generally accompanied by an increased wakefulness after sleep onset (WASO) (6–10,12). Conversely, sleep efficiency typically remains unaffected by altitudes up to 4000 m (4,6,7,9,10,14–16), but decreases by 10% to 20% at 4000 m (6,16) and higher (12).

Sleep disturbances may have both direct and indirect implications for athletic performance. This is evidenced by studies showing that a single night of restricted sleep (i.e., sleep time reduction of 2 to 4 h) increases cardiac stress during and after exercise on the next day (17). Furthermore, ~5 d of disturbed sleep has been shown to suppress myofibrillar and sarcoplasmic protein synthesis rates (18,19), to impair insulin sensitivity (20), and to reduce mitochondrial respiratory function (19). Although some studies reported no impact of sleep restriction down to 2 h per night on next-day exercise performance (17,21), other studies indicated that a single night of ~2 to 3 h of sleep deprivation is sufficient to decrease 6-s peak cycling power output and both 3- and 20-km cycling time-trial performance (5,22). Nevertheless, it is currently unknown if the more subtle differences in sleep quality and quantity that are expected to occur upon a single night in hypoxia also impair next-day exercise performance.

The precise underlying mechanisms of hypoxia-induced sleep impairments are currently unknown. Yet, they are most likely caused by hypoxemia (11,15,23,24). Interestingly, we previously showed that increasing blood ketone bodies (KB), via oral ketone ester (KE) ingestion, attenuated the drop in blood (as well as muscle and brain) oxygenation after 3 to 4 h in hypoxia (25,26). Furthermore, we identified that KE intake postexercise and presleep beneficially impacted sleep after strenuous, late evening exercise in normoxic conditions. This was evidenced by an inhibition of late evening exercise-induced decreases in REM sleep and WASO, and a 2% improvement in sleep efficiency (27).

These data raise the question of whether presleep KE ingestion may be an effective strategy to attenuate hypox(em)ia-induced sleep dysregulations. Against this background, this study aims to identify the impact of a single night in hypoxia on (i) nocturnal sleep measured using gold standard polysomnography (PSG) and (ii) next-day exercise performance, and to (iii) explore the potential of KE supplementation to counteract potential performance or sleep dysregulations under these conditions.

METHODS

Ethical Approval and Participants

This research was registered at www.clinicaltrials.gov (NCT06060093) and approved by the Ethics Committee Research UZ/KU Leuven (B3222022001041). Potential participants were medically screened and provided written informed consents. Candidates' sleep quality was screened using the Pittsburgh Sleep Quality Index (PSQI) to guarantee adequate baseline sleep quality as defined by the National Sleep Foundation (i.e., sleep efficiency above 85%). Participants working in late-night shifts, as well as extreme morning and evening chronotypes as determined by the Horne–Östberg questionnaire, were excluded. All 13 healthy male participants that were included reported good sleep quality and no indications for sleep disorders (PSQI score <5). None of them showed indications for psychological or neurological disorders, such as depression (Beck's Depression questionnaire) or anxiety (Beck's Anxiety questionnaire). All participants were active in cycling, endurance running, or triathlon. Further exclusion criteria included smoking, exposure to altitudes >1500 m during the 3 months preceding the study, or a history of altitude-sensitive pathologies. From the 13 participants that were initially included, one dropped out because of a COVID-19 infection and another one was excluded from the data analyses because of a baseline sleep quality drastically below the predefined inclusion criteria (i.e., sleep efficiency of 78%). Therefore, data analyses were performed on 11 participants [age: 24 ± 4 yr; height: 181.3 ± 7.5 m; body mass: 73.2 ± 9.2 kg; physical activity: 8 ± 3 $h \cdot wk^{-1}$, $\dot{V}O_2max$: 61.5 ± 8.8 $mL \cdot kg^{-1} \cdot min^{-1}$ (mean ± SD)]. Participants were located within Tier 2 according to the Athlete Classification Framework (28).

General Study Design

This randomized, double-blind, placebo-controlled, cross-over study consisted of three experimental sessions. These sessions were designed to mimic a live-high, train-low setup, and all included an identical training session in normoxia whereafter participants either spend the night in (i) normoxia (N_{PL}) or (ii) normobaric hypoxia (~3000 m of simulated altitude) while receiving a placebo (H_{PL}), or in (iii) hypoxia while receiving KE (H_{KE}, Fig. 1). After the night in either hypoxia or normoxia, participants performed an all-out 30-min time trial ($TT_{30'}$) to evaluate exercise performance. Physiological measurements were performed at rest throughout both days of the protocol. This design allowed us to evaluate both (i) the impact of sleeping in hypoxia versus normoxia, and (ii) the potential of KE to improve sleep in hypoxia. All experimental sessions were conducted in a normobaric hypoxic facility (Van Amerongen CA Technology, Tiel, the Netherlands) at the Bakala Athletic Performance Center (Leuven, Belgium). The experimental sessions were separated by a 1-wk wash-out period, and experimental protocols and timings were identical for all three sessions.

Preliminary Testing and Familiarization Sessions

Two weeks before the first experimental session, participants performed a familiarization session consisting of a graded exercise test on a cycling ergometer (Avantronic Cyclus II, Leipzig, Germany) to assess both their lactate threshold (LT) and maximal oxygen uptake rate ($\dot{V}O_2max$). For the LT determination,

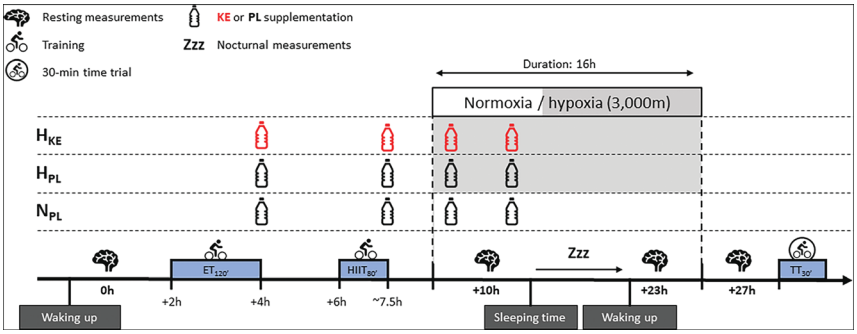

FIGURE 1—Schematic representation of the experimental protocol that was designed to mimic a live-high train-low (LHTL) strategy. In a double-blind, randomized, crossover design, 11 participants completed three experimental sessions each involving a 2-day protocol. On day 1, subjects performed two exercise training sessions [120 min endurance training ($ET_{120'}$) and 80 min high intensity interval training ($HIIT_{80'}$)] in normoxia followed by a night either in normoxia (N) or at a simulated altitude of 3000 m (H). After each training session and before sleep, participants received either placebo (N_{PL} and H_{PL}) or KE (H_{KE}) supplements. On day 2, exercise performance was evaluated by means of a 30-min all-out time trial ($TT_{30'}$). Sleep quality and architecture were assessed via PSG. Physiological measurements were performed in a resting state at baseline, after 2 h in hypoxia or equivalent in normoxia, immediately upon waking up and 2 h after return to normoxia to assess blood oxygen saturation, cerebral and muscular oxygenation status, ventilatory gas exchange, HR, and HRV. In addition, before every resting measurement, as well as immediately before sleep time, a capillary blood sample was collected for determination of acid–base balance and blood gasses.

initial workload was set at 70 W and increased by 40 W per 8 min. Capillary blood samples were obtained every 4 min for determination of blood lactate concentration (Lactate Pro2; Arkray, Amstelveen, the Netherlands), with LT being defined as the lowest workload provoking a 1 mM blood [lactate] increase within the same stage (27,29,30). After 15 min of active recovery at 70 W, participants started a second incremental cycling test (100 W + 25 W/30 s) until volitional exhaustion. Throughout this test, oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) rates were measured continuously using indirect calorimetry (Cortex Metalyzer 3B; Cortex, Leipzig, Germany), and $\dot{V}O_2$max was calculated as the highest $\dot{V}O_2$ over a 30-s period.

In the 2 wk before the first experimental session, participants performed the full experimental protocol twice in normoxia to get accustomed to the experimental procedures, exercise protocols, sleeping facility, and instrumentation. Starting from the first familiarization session, participants were asked to maintain a stable sleep schedule until the final experimental session. This included the requirement to sleep 7 to 9 h per night, with bedtime between 10 and 12 PM and wake time between 6 and 8 AM. Compliance to a stable sleep–wake schedule was assessed throughout the entire study period via a sleep diary and actigraphy (Actigraph wGT3X-BT; ActiGraph LLC, Pensacola, FL). Moreover, sleep quality of the night before each experimental session was also assessed using the St. Mary's Sleep questionnaire.

Experimental Sessions

After waking up at their individually predetermined time, participants arrived in a fasted state between 7 and 9 AM at the testing facility (exact timings were replicated for each session). They performed a 2-h morning endurance training session ($ET_{120'}$) on a cycling ergometer (Tacx Neo Smart, Wassenaar, the Netherlands) 2.5 h after consuming a standardized breakfast (see *Dietary standardization*). $ET_{120'}$ consisted of eight consecutive 15-min intervals with workload alternating between 60% and 80% of LT. 1.5 h after lunch time, participants completed an 80-min high intensity interval training ($HIIT_{80'}$) on a cycling ergometer (Avantronic Cyclus II). $HIIT_{80'}$ consisted of a 10-min warm up at 70% of LT, followed by 10 repetitions of 3 min at 120% of LT interspersed by 4-min active recovery at 50% of LT. Thirty minutes after $HIIT_{80'}$ (~5 h before sleep time), participants entered their individual "hotel room" that was set at either hypoxia (H_{PL} and H_{KE}; F_IO_2: 14.5% O_2, P_IO_2: ~110 mm Hg, ~3000 m simulated altitude) or normoxia (N_{PL}; F_IO_2: 20.9% O_2, P_IO_2: ~160 mm Hg). Subsequently, participants resided for 16 h under these environmental conditions, with sleep being measured in each condition using PSG. After 16 h of hypoxia/normoxia, participants returned to normoxia and exercise performance was assessed 3.5 h later by means of an all-out 30-min time trial ($TT_{30'}$). In addition, physiological measurements were performed at rest at the following time points: (i) before $ET_{120'}$ (baseline, 0 h), (ii) after 2 h in hypoxia or equivalent in normoxia (+10 h), (iii) immediately upon waking up (+23 h), and (iv) 2 h after returning to normoxia (+27 h). These measurements included determination of heart rate (HR); HR variability (HRV); ventilatory parameters; and blood, brain, and skeletal muscle oxygenation.

Supplementation Protocol

In a randomized order, participants received 25 g of either a KE [H_{KE}, 96% (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate; KetoneAid Inc., Falls Church, VA] or a placebo (N_{PL} and H_{PL}, details hereinafter) supplement immediately after (i) $ET_{120'}$ and (ii) $HIIT_{80'}$, (iii) after 1.5 h in hypoxia (or equivalent in normoxia), and (iv) 30 min before sleep. Hence, in H_{KE}, participants received a total dose of 100-g KE (1.39 ± 0.17 $g \cdot kg^{-1}$ body weight) to intermittently elevate blood KB postexercise and throughout the first part of the night. In N_{PL} and H_{PL}, participants received a total of 100 g of a taste- and viscosity-matched, inert placebo consisting of 12.5% w/v collagen (6d Sports Nutrition, Oudenaarde, Belgium) and 1 mM bitter

sucrose octaacetate (Sigma-Aldrich, Bornem, Belgium) dissolved in water. In total, caloric intake via the KE supplements was ~1960 kJ versus ~190 kJ for the placebo supplements. Our decision for an inert low-caloric placebo drink aimed to exclude potential effects on sleep from increased carbohydrate intake or slightly increased KB production associated with increased fat intake. Randomization was performed by a researcher who was otherwise not involved in the study.

Dietary Standardization

The evening before each experimental session, participants consumed a standardized carbohydrate-rich dinner at home (~5600 kJ; 69% carbohydrate, 16% fat, 15% protein). The meal was provided by the researchers and identical before each session. If participants did not finish this meal before the first session, the leftovers were measured and the meal was replicated before the following sessions. Water consumption was allowed *ad libitum* until arrival at the facility the next morning, and was replicated for each session. After participants arrived at the testing facility in a fasted state, they consumed a standardized breakfast (~4200 kJ; 68% carbohydrate, 21% fat, and 11% protein). Subsequently participants received a carbohydrate-rich snack (~660 kJ; 92% carbohydrate, 3% fat, and 5% protein) 1 h before $ET_{120'}$, and received 60-g carbohydrates per hour during $ET_{120'}$ via isotonic drinks and carbohydrate-rich snacks (6d Sports Nutrition, Oudenaarde, Belgium). A light lunch (~4150 kJ; 74% carbohydrate, 25% fat, and 14% protein) was provided 1.5 h before $HIIT_{80'}$, and participants received a high-carbohydrate, high-protein recovery shake (60-g carbohydrate, 30-g protein; 6d Sports Nutrition, Oudenaarde, Belgium) immediately after $HIIT_{80'}$. After 30 min in hypoxia/normoxia, a standardized dinner (~3250 kJ; 69% carbohydrate, 5% fat, and 26% protein) was served while participants received a light snack (~1700 kJ of which 69% carbohydrates, 15% protein, 16% fat) 2 h before sleep time to prevent participants from going to bed hungry. Hence, total caloric intake on the first day—excluding the KE supplements—was ~15,020 kJ. The next morning, participants received an identical breakfast as on the first day and received a carbohydrate-rich snack (cfr. before $ET_{120'}$) 30 min before $TT_{30'}$.

Nocturnal Measurements

Polysomnography. A digital amplifier (V-amp; Brain Products, Gilching, Germany) was used to record sleep quality and quantity during the experimental sessions, and data were digitized at a sampling rate of 1000 Hz. According to the international 10 to 20 system, electroencephalographic (EEG) recordings were made from Fz, Cz, Pz, Oz, C3, C4, A1, and A2, where the A2 electrode served as the reference electrode and A1 as a backup reference. A ground electrode was positioned in the middle of the forehead. Eye movements (vertical/horizontal) were recorded with electrooculographic (EOG) electrodes above and under the right eye and with electrodes attached to the outer cantus of both eyes. A 0.1 Hz low cut-off filter and a 30 Hz high cut-off filter were applied to both EEG and EOG data. Submental muscle tone and movements were assessed with a electromyogram (EMG) of the chin, recorded with a low cut-off filter of 10 Hz and a high cut-off filter of 200 Hz. Electrical noise was filtered out with a 50 Hz notch filter. A blinded, independent, certified sleep technician (Sleep Well PSG, Canada) visually scored night recordings after the Rechtschaffen & Kales guidelines, in conjunction with the AASM guidelines, in 30-s epochs. Bandpass filters at 0.3 to 35 Hz, 0.3 to 30 Hz, and 10 to 100 Hz were used for EEG, EOG, and EMG signals, respectively. The assessed sleep variables were (i) total sleep time; (ii) WASO; (iii) total non-rapid eye movement (NREM) and REM sleep; (iv) total N1, N2, and REM and SWS sleep; (v) sleep onset, sleep onset to N2, SWS, and REM sleep; (vi) sleep efficiency (total sleep time/time in bed), and (vii) amount of awakenings.

Sleep event detection. EEG data were preprocessed in BrainVision Analyzer (Brain Products GmbH). After applying a 0.1 to 30 Hz bandpass filter, data were transferred to a Python environment (version 3.10.5). Detection of both "slow waves" and "sleep spindles" was performed according to previously described guidelines (31). For each channel, the densities of slow waves and sleep spindles were averaged across the channels for N2 and SWS phases.

Oxygen saturation and HR. During the participants' registered sleep time, blood oxygen saturation (SpO_2) and HR were continuously assessed using pulse oximetry (Nellcor PM10N; Medtronic, Minneapolis, MN). These data were used to calculate both average and minimum HR, the minimum SpO_2, and the average nocturnal SpO_2 both over the entire night as well as within 10% epochs of the night. Furthermore, variation in SpO_2 was assessed through a coefficient of variation (CV) for every hour ($CV_i = SD_i/mean_i$ with i = the hour of the night) in agreement with an earlier study (32). CV_i was calculated given earlier evidence that periodic breathing causes fluctuations in nocturnal oxygen saturation in preterm infants (33).

Resting Measurements

All resting measurements were performed by the same trained researcher. Measurements were performed throughout a 10-min time window and with the participants being in supine position for at least 10 min before the start of the first measurement. Data for all parameters are presented as the average values of the last minute.

Blood and tissue oxygenation status. Blood oxygen saturation (SpO_2) was assessed using a pulse oximeter (Nellcor PM10N, Medtronic) with an infrared sensor placed ~2 cm above the left eyebrow and with sampling frequency set at 2 Hz. Cerebral status and skeletal muscle oxygenation status were assessed by means of tissue oxygenation index (cTOI and mTOI, respectively) using near-infrared spectroscopy (NIRS). 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). A constant penetration depth of ~2 cm into the muscle/brain tissue was ensured by inserting the emitter and detector probes in a dark-colored rubber

spacer, maintaining a fixed interoptode distance of 4 cm. These spacers were attached to the participants using an elastic nontransparent bandage and double-sided adhesive tape to prevent displacement or interference from external light. Before each experimental session, the participants' skin was shaved and cleaned to exclude any signal disturbance by hair or impurities. Moreover, exact intersession replication was guaranteed by marking the contour lines of the rubber spacer on the skin. Participants were asked to preserve and refresh these marks during the washout period to maintain this position during the following sessions. After experimental data collection, NIRS data were preprocessed (Matlab R2023a; The Mathworks, Natick, MA) over 1-min long time chunks using a fourth-order Butterworth filter with a cut-off frequency of 0.05 Hz (26).

HR and HRV. Resting HR and RR-peak intervals were measured using a Polar H10 (Polar, Kempele, Finland). HRV was analyzed by using the percentage of adjacent NN intervals that differ by more than 50 ms (pNN50), the root mean square of successive differences (RMSSD), and absolute power of the high-frequency band (HF) using Kubios (Kubios HRV Standard 3.5.0; Kubios Oy, Kuopio, Finland).

Ventilatory gas exchange measurements. Indirect calorimetry (Cortex Metalyzer 3B) was used to measure breath-by-breath gas exchange data [*i.e.,* minute ventilation ($\dot{V}E$), oxygen uptake rate ($\dot{V}O_2$), and carbon dioxide production rate ($\dot{V}CO_2$)].

Exercise Performance

After returning to normoxia on the second day, participants performed $TT_{30'}$, which was preceded by a 15-min warm-up at 70% of LT on a cycling ergometer (Avantronic Cyclus II). Participants were instructed to achieve an average power output as high as possible and were allowed to voluntarily change the applied workload every 5 min in the initial 25 min, and every 1 min during the final 5 min. During $TT_{30'}$, they could only see a countdown timer and were blinded in terms of their HR and power output. The starting workload was determined during the familiarization sessions, where initial power in the first session was set at the LT and in the second as the average power output of the first $TT_{30'}$. In turn, the average of the second $TT_{30'}$ functioned as the starting workload of both experimental $TT_{30'}$.

Capillary Blood, Urine Sampling, and Analyses

Capillary samples. Before breakfast (baseline, 0 h), 30 min after every supplement ingestion (+5.5 h, +9 h and +11 h and +13 h), and immediately upon waking up on the second morning (+23 h), a capillary blood sample was obtained for immediate determination of D-ß-hydroxybutyrate by a researcher who was not otherwise involved in the study (GlucoMen Areo 2K-meter with β-ketone sensor strips; A. Menarini Diagnostics, Firenze, Italy). In addition, 70-μL capillary blood was collected from a hyperemic earlobe into a capillary tube (safeCLINITUBE; Radiometer Medical ApS, Copenhagen, Denmark) before every resting measurement (baseline, +10 h, +23 h and +27 h) and immediately before sleep (+13 h). After immediate mixing for 10s, samples were analyzed for acid–base balance, p50, and pCO_2 (ABL90 FLEX analyzer, Radiometer Medical ApS).

Urine samples. Immediately before sleep time, participants emptied their bladder and nocturnal urine was collected until wake-up in urine flasks prepared with 10 mL hydrochloric acid. A small volume (~10 mL) of urine was frozen (−80°C) for subsequent analyses of adrenaline, noradrenaline, and dopamine using a commercially available ELISA kit (BA E-6600; LDN, Nordhorn, Germany) within 4 wk after the final experimental session.

Statistical Analyses

All statistical analyses were performed in GraphPad Prism version 10.1.2 (GraphPad Software, La Jolla, CA). Before statistical testing, normal distribution of the data was evaluated and confirmed with the D'Agostino-Pearson normality test for SpO_2 and HR during the night as well as power output and HR during $TT_{30'}$. One way analysis of variance (ANOVA) was used to evaluate differences between conditions for measurements obtained at a single time point during each session. Data collected at multiple time points within a given experimental session were analyzed by means of a two-way repeated measures ANOVA. A Geisser–Greenhouse correction was applied whenever the assumption of sphericity was violated (Mauchly's test, JASP version 0. 18.1; JASP Team, Amsterdam, the Netherlands). In case of a significant main or interaction effect, *post hoc* analyses were performed using Šidák's correction. For significant *post hoc* analyses, effect sizes (Cohen's *d*) and 95% confidence intervals (CI) were reported for the relevant differences. 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, and statistical significance was defined as $P < 0.05$. An *a priori* sample size calculation was performed based on the effect size of KE on sleep efficiency (i.e., primary outcome of the current study) that was derived from our previous study investigating the effect of KE during sleep in normoxia (27). Therefore, the effect size was calculated using the η_p^2 between the EXCON and EXKE conditions from this earlier study (27). Power analysis (G*Power version 3.1.9.7) indicated that a sample size of 6 is required to establish a significant effect on sleep efficiency (η_p^2: 0.395; effect size $f = 0.81$; α error: 0.05; power: 0.80; number of groups: 3; number of measurements: 3; correlation among repeated measures: 0.5; nonsphericity correction: 1; ANOVA: repeated measures, within factors).

RESULTS

Blood βHB Concentrations

A time × condition effect was observed for blood [βHB] ($P < 0.001$, Fig. 2). Baseline concentrations were low (~0.4 mM) in all conditions, and concentrations remained low (~0.4 to 0.5 mM) throughout the entire protocol in N_{PL} and H_{PL}. Conversely, blood [βHB] consistently increased to 2 to 3 mM (range: 1.4 to 4.7 mM) 30 min after ingestion of each KE

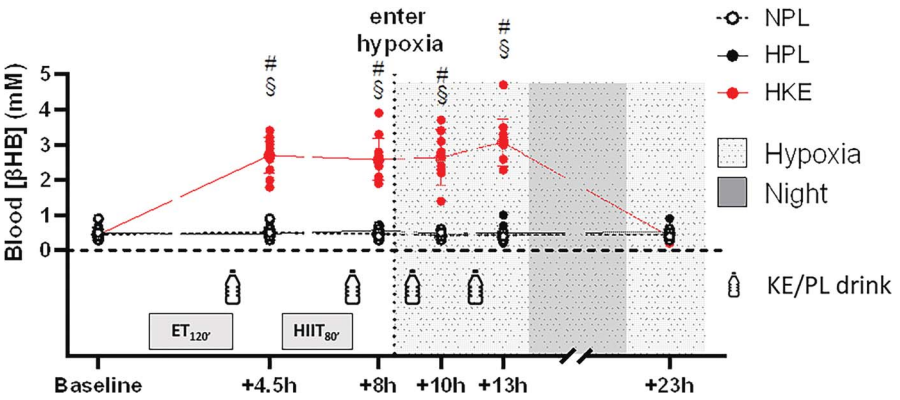

FIGURE 2—Blood D-β-hydroxybutyrate concentrations ([βHB]) are presented in response to ingestion of KE or placebo (PL) supplements during experimental sessions including a night (dark gray zone) either in normoxia (N_{PL}, black, open circles) or at a simulated altitude of 3000 m (*gray dotted zone*; H_{PL}, *black*, and H_{KE}, *red full circles*). Blood [βHB] was assessed at baseline, 30 min after the morning endurance training ($ET_{120'}$; +5.5 h) and the afternoon high-intensity interval training ($HIIT_{80'}$; +9 h), as well as after 2 h in hypoxia/normoxia (+11 h), immediately before sleep (+13 h), and upon waking up (+23 h). Mean (line) ± SD (whiskers), as well as individual values are shown for $n = 11$. $^{§}P < 0.05$ versus N_{PL}; $^{\#}P < 0.05$ versus baseline for indicated condition.

supplement. Upon waking up the second day of the protocol, blood [βHB] had returned to baseline values in all conditions.

Sleep Architecture

Time in bed was kept constant for a given subject across all conditions and was on average 512 ± 25 min. Compared with N_{PL}, H_{PL} decreased sleep efficiency by 3% ($P = 0.037$ for H_{PL} vs N_{PL}, $d = -0.73$, 95% CI of difference = 0.1% to 4.8%, Fig. 3A). This reduced sleep efficiency was mediated by a doubling (+~17 min) of WASO ($P = 0.049$ for H_{PL} vs N_{PL}, $d = 0.89$, CI = −34 to 0 min, Fig. 3B), increased number of arousals ($P = 0.027$ for H_{PL} vs N_{PL}, $d = 0.58$, CI = −77 to −5, Fig. 3C) while sleep onset latency ($P = 0.316$ for main effect, Fig. 3D), and latencies of N2, SWS, and REM phases remained unaffected (all $P > 0.29$ for main effects, data not shown). The reduced sleep efficiency in H_{PL} versus N_{PL} was accompanied by a ~22% reduction in SWS ($P = 0.002$ for H_{PL} vs N_{PL}, $d = -1.37$, CI = 8 to 37 min, Fig. 3E), and a ~39% increase in N1 duration ($P = 0.034$ for H_{PL} vs N_{PL}, $d = 0.65$, CI = −14 to −1 min, Fig. 3F), whereas N2 phase ($P = 0.271$ for N_{PL} vs H_{PL}, Fig. 3G) and REM sleep duration ($P = 0.153$ for main effect, Fig. 3H) remained unaffected. KE did not affect any of the hypoxia-induced dysregulations because all parameters were similar between H_{PL} and H_{KE} (all $P > 0.340$ for H_{KE} vs H_{PL}). *Post hoc*, we confirmed that no order effect was present for sleep efficiency ($P = 0.538$). Densities of both sleep spindles and slow waves during N2 ($P = 0.082$ and $P = 0.307$ for main effects,

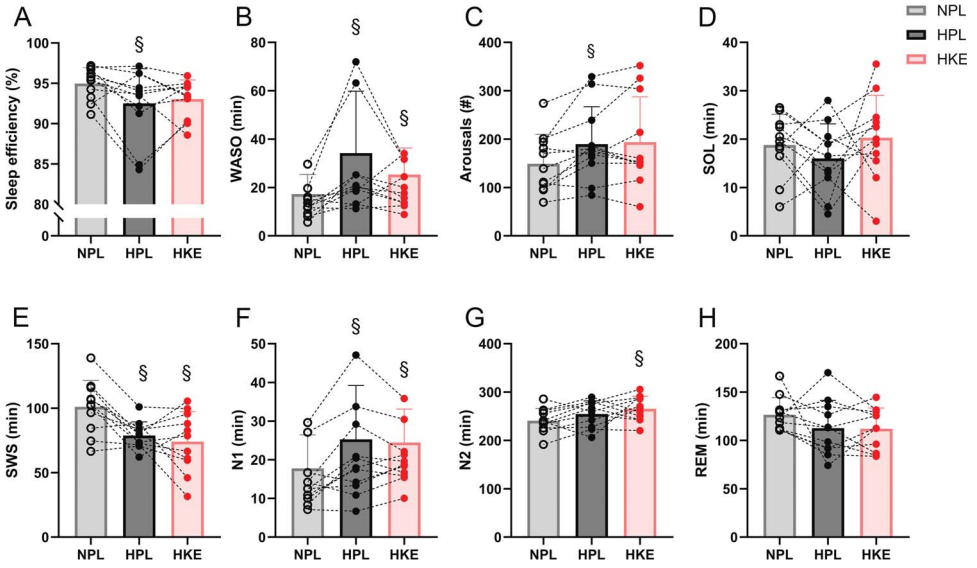

FIGURE 3—Data of polysomnographic (PSG) recordings are shown for participants that completed three experimental sessions in which they received either KE or placebo (PL) supplements. They spent the night either in normoxia (N_{PL}) or at a simulated altitude of 3000 m (H_{PL} and H_{KE}). During the night, participants' sleep was recorded using PSG. Mean (bar plots) ± SD (whiskers) and individual values are shown for (A) sleep efficiency, (B) WASO, (C) the amount (#) of arousals per night, and (D) sleep onset latency (SOL). Moreover, sleep stage durations are shown for (E) SWS, (F) N1 phase, (G) N2 phase, and (H) REM sleep. Data are reported for 11 participants, and data points are connected within each participant. $^{§}P < 0.05$ versus N_{PL}.

respectively) and SWS ($P = 0.752$ and $P = 0.782$ for main effects, respectively) were unaffected throughout the experimental sessions (data not shown).

Subjective Sleep Quality

A main effect was observed for the following questions in the St. Mary's sleep questionnaire; *"How deep was your sleep?"* (N_{PL}: 5 ± 1, H_{PL}: 4 ± 1, H_{KE}: 4 ± 1; $P = 0.007$), *"How many times did you wake up?"* (N_{PL}: 3 ± 1, H_{PL}: 4 ± 2, H_{KE}: 4 ± 1; $P = 0.007$), *"How clear-headed did you feel after getting up this morning?"* (N_{PL}: 4 ± 1, H_{PL}: 2 ± 1, H_{KE}: 3 ± 1; $P = < 0.001$), *"How satisfied were you with last night's sleep?"* (N_{PL}: 4 ± 1, H_{PL}: 3 ± 1, H_{KE}: 3 ± 1; $P = 0.015$). *Post hoc* analyses indicated that, compared with N_{PL}, participants in H_{PL} perceived that they slept less deep ($d = -1.28$), woke up more times during the night ($d = 0.76$), felt less clear headed after getting up ($d = -1.41$), and were less satisfied with their night's sleep ($d = -0.99$). Except for a more clear-headed feeling upon waking up in H_{KE} ($P = 0.012$ for H_{KE} vs H_{PL}, $d = 0.94$), no differences were observed between H_{PL} and H_{KE}. No effects were observed for the questions *"How much sleep did you have last night?"* (N_{PL}: 8 ± 1, H_{PL}: 7 ± 1, H_{KE}: 8 ± 1; $P = 0.099$), *"How much sleep did you have during the day, yesterday?"* (N_{PL}: 0 ± 0, H_{PL}: 0 ± 0, H_{KE}: 0 ± 0), *"How well did you sleep last night?"*, (N_{PL}: 4 ± 1, H_{PL}: 3 ± 1, H_{KE}: 3 ± 1; $P = 0.051$), *"Were you troubled by waking early and being unable to get off to sleep again?"*, (N_{PL}: 1 ± 1, H_{PL}: 0 ± 1, H_{KE}: 1 ± 0; $P = 0.123$), and *"How much difficulty did you have in getting off to sleep last night?"*, (N_{PL}: 1 ± 1, H_{PL}: 2 ± 1, H_{KE}: 1 ± 0; $P = 0.224$).

Nocturnal Oxygen Saturation and HR

At the start of the night, SpO_2 was 9% lower in H_{PL} compared with N_{PL} ($P < 0.001$, $d = -3.18$, CI = 7% to 12%, Fig. 4A). Throughout the first half of the night, SpO_2 gradually decreased

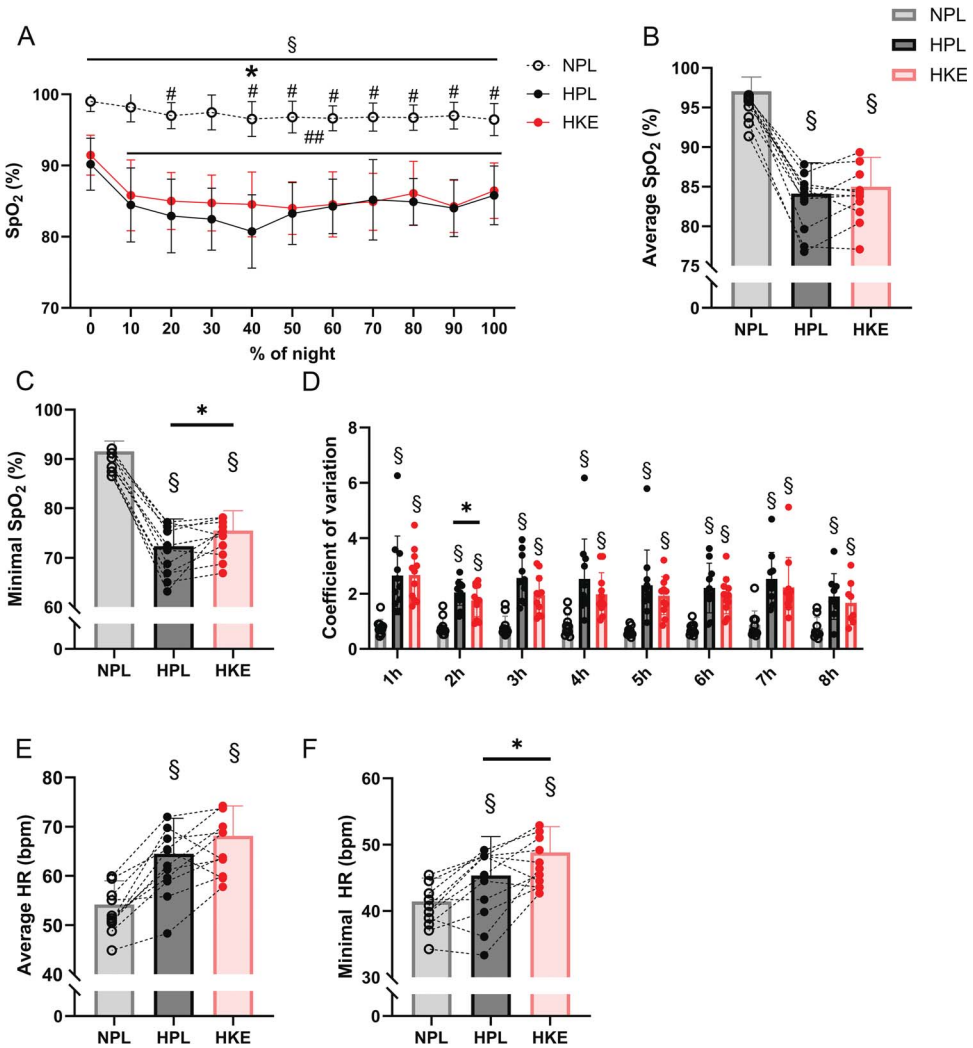

FIGURE 4—Nocturnal measurements of blood oxygen saturation (SpO_2) and HR are shown for participants that completed three experimental sessions in which they received either KE or placebo (PL) supplements. They spent the night either in normoxia (N_{PL}) or at a simulated altitude of 3000 m (H_{PL} and H_{KE}). Mean (circles) ± SD (whiskers) is shown for (A) SpO_2 throughout the percentual duration of the night (%). Mean (bar plots) ± SD (whiskers), and individual values are shown for (B) average and (C) minimum nocturnal SpO_2 and (D) coefficient of variance of SpO_2 per hour of the night, as well as (E) average and (F) minimum nocturnal HR. Data are reported for 11 participants, and individual values are connected within each participant. $^{\S}P < 0.05$ versus N_{PL}; $^{\#}P < 0.05$ versus baseline; $^{\#\#}P < 0.05$ versus baseline for H_{PL} and H_{KE}; $^{*}P < 0.05$ for H_{KE} versus H_{PL}.

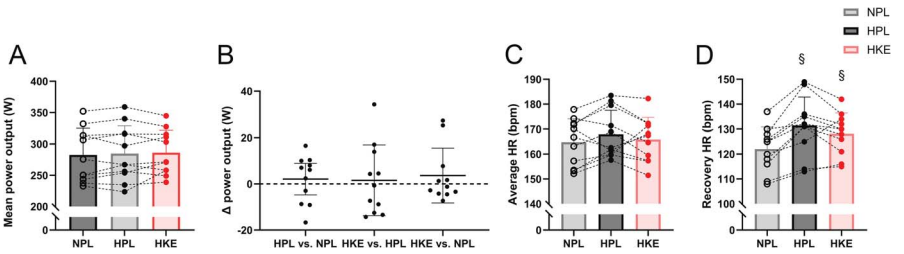

FIGURE 5—Exercise performance and HR data are shown for participants that completed three experimental sessions in which they received either KE or placebo (PL) supplements during the first day. After spending the night in either in normoxia (N_{PL}) or at a simulated altitude of 3000 m (H_{PL} and H_{KE}), exercise performance was assessed by an all-out 30-min time trial ($TT_{30'}$). Mean (bar plots) ± SD (a, c, d, whiskers) or mean (horizontal indication) ± 95% CI (b, whiskers), as well as individual values are shown for (A) mean power output, (B) the individual differences in mean power output between all experimental conditions, and (C) average HR during $TT_{30'}$ and (D) HR after 5 min of passive recovery. Data are reported for 11 participants, and individual values are connected within each participant. $^{§}P < 0.05$ versus N_{PL}.

in all conditions, reaching minimum SpO_2 levels after ~220 min in bed or ~44% of sleep for all conditions ($P = 0.954$ and $P = 0.921$, respectively, data not shown). Because of both lower baseline values and a steeper reduction throughout the night in H_{PL} versus N_{PL}, minimum and average SpO_2 were 20% and 13% lower, respectively, in H_{PL} compared with N_{PL} ($P < 0.001$ for both, $d = -4.63$ and $d = -4.26$, CI = 16% to 22% and 11% to 15%, Fig. 4B and C, respectively). KE ingestion alleviated the gradual drop in SpO_2 throughout the first part of the night (Fig. 4A). Hence, SpO_2 after 40% of the night and minimum SpO_2 were both 4% higher in H_{KE} versus H_{PL} ($P = 0.029$ and $P = 0.048$, $d = 0.78$ and $d = 0.64$, CI = −7% to 0% and −6% to 0%, Fig. 4A and B, respectively). Conversely, average nocturnal SpO_2 was similar between H_{KE} and H_{PL} ($P = 0.519$ vs H_{PL}, Fig. 4C).

H_{PL} strongly increased the CV of SpO_2 during all hours of the night compared with N_{PL} ($P < 0.002$ for main effect, Fig. 4D). This increase in CV was attenuated by KE ingestion during the second hour of the night ($P = 0.008$ vs H_{PL}), but not at any of the other time points. Compared with N_{PL}, H_{PL} increased minimum and average nocturnal HR by ~10% and ~20%, respectively ($P = 0.008$ and $P < 0.001$ for H_{PL} vs N_{PL}, $d = 0.81$ and $d = 1.68$, CI = −7 to −1 bpm and −14 to −6 bpm, Fig. 4E and F, respectively). KE further increased minimum nocturnal HR by ~8% ($P = 0.019$ for H_{KE} vs H_{PL}, $d = 0.69$, CI = −6 to −1 bpm) and tended to further elevate average nocturnal HR ($P = 0.079$ for H_{KE} vs H_{PL}).

Exercise Performance

Mean power output during $TT_{30'}$ was similar between all conditions at ~285 W ($P = 0.454$, 95% CI: −5 to +9 W for N_{PL} vs H_{PL} and −9 to +12 for H_{KE} vs H_{PL}) (Fig. 5A and B). *Post hoc*, we confirmed that no order effect was present for mean power output during $TT_{30'}$ ($P = 0.271$). Moreover, we previously identified that the power output during this $TT_{30'}$ protocol had a CV of 2.1%. Also, average HR during $TT_{30'}$ (Fig. 5C) was similar between all conditions ($P = 0.251$). However, after 5 min of passive recovery, participants' HR remained ~6 to 9 bpm higher in H_{PL} and H_{KE} compared with N_{PL} ($P < 0.001$ for H_{PL} vs N_{PL}, $d = 0.93$, CI = −13 to −6 bpm, Fig. 5D).

Resting Measurements—Oxygen Status, HR, and HRV

A time × condition effect was detected for SpO_2 ($P < 0.001$, Table 1). Compared with N_{PL}, SpO_2 dropped to a similar extent in H_{PL} and H_{KE} both at +10 h ($P < 0.001$ for H_{PL} vs N_{PL}, $d = -3.82$, CI = 8% to 14%) and +23 h ($P < 0.001$ for H_{PL} vs N_{PL}, $d = -3.80$, CI = 7% to 10%). Conversely, both cTOI and mTOI remained stable at ~73% and ~67%, respectively, in all experimental conditions (data not shown). For HR, a time × condition effect was found ($P < 0.001$, Table 1). *Post hoc* analyses indicated that HR was ~14% higher in H_{PL} versus N_{PL} at +10 h ($P < 0.001$, $d = 1.13$, CI = −13 to −6 bpm), and even ~20% higher at +23 h ($P < 0.001$, $d = 1.21$, CI = −14 to −6 bpm).

TABLE 1. Effect of hypoxia (H_{PL} vs N_{PL}) and KE ingestion (H_{KE} vs H_{PL}) on blood oxygen saturation (SpO_2), HR, and HRV.

	N_{PL}	H_{PL}	H_{KE}
SpO_2 (%)			
Baseline	97.4 ± 1.7	98.5 ± 1.5	98.7 ± 1.3
+10 h	98.2 ± 1.1	87 ± 4$^{§,\#}$	88.5 ± 4.5$^{§,\#}$
+23 h	98.1 ± 2.1	89.2 ± 2.6$^{§,\#}$	90.8 ± 3.3$^{§,\#}$
+27 h	98.2 ± 1.7	97.9 ± 1.7	98.7 ± 1
HR (bpm)			
Baseline	60.1 ± 6.1	60.7 ± 5.1	57.6 ± 6.1
+10 h	65.6 ± 7.8 $^{\#}$	74.9 ± 8.6$^{§,\#}$	84.8 ± 7$^{§,\#,*}$
+23 h	51.4 ± 6.3 $^{\#}$	61.3 ± 9.7§	63.6 ± 8.4$^{§,\#}$
+27 h	55.2 ± 6.4 $^{\#}$	56.9 ± 6.9	58.2 ± 7.8
pNN50 (%)			
Baseline	43.4 ± 21.6	45.2 ± 24.8	52.5 ± 23.2
+10 h	34.4 ± 23.5	14.1 ± 19.2$^{§,\#}$	4.2 ± 7.6$^{§,\#}$
+23 h	59.5 ± 24.9	38.3 ± 21.3§	22.3 ± 17.1$^{§,*,\#}$
+27 h	47.4 ± 23.5	50.1 ± 27.0	51.9 ± 23.2
RMSSD (ms)			
Baseline	75.4 ± 41.7	74.2 ± 29.3	86.7 ± 37.4
+10 h	62.7 ± 43.1	32.1 ± 22.2$^{§,\#}$	21.3 ± 20.6$^{§,\#}$
+23 h	94.2 ± 38.4	70.3 ± 36.2§	50.8 ± 28.2$^{§,\#}$
+27 h	88.9 ± 38.1	96.8 ± 51.6	87.8 ± 38.5
HF (log)			
Baseline	7.42 ± 1.00	7.46 ± 0.72	7.41 ± 1.00 $^{\#}$
+10 h	7.04 ± 1.00	5.67 ± 1.19$^{§,\#}$	4.75 ± 1.56$^{§,\#}$
+23 h	7.07 ± 0.99	7.14 ± 0.91	6.46 ± 1.16*
+27 h	7.48 ± 1.34	7.5 ± 1.25	7.62 ± 1.05

During a randomized, cross-over protocol, participants received either KE or placebo (PL) supplements and spent the night either at a simulated altitude of 3000 m (H_{PL} and H_{KE}) or in normoxia (N_{PL}). Participants' blood oxygen saturation ($SpSO_2$), HR, and HRV were assessed at baseline, after 2 h in hypoxia/normoxia (+10 h), immediately upon waking up in hypoxia/normoxia (+23 h) and 2 h after returning to normoxia (+27 h). Mean values ± SD ($n = 11$) are shown for SpO_2, HR, the percentage of adjacent NN intervals that differ by more than 50 ms (pNN50), the RMSSD, and the absolute power of the high-frequency band (HF). $^{§}P < 0.05$ versus N_{PL}; $^{*}P < 0.05$ versus H_{PL}; $^{\#}P < 0.05$ versus baseline.

KE ingestion further increased resting HR at +10 h ($P < 0.001$ for H_{KE} vs H_{PL}, $d = 1.26$, CI = −23 to −16 bpm). An interaction effect was observed for all measured HRV indices (pNN50, $P = 0.002$; RMSSD, $P < 0.001$; HF, $P = 0.005$; Table 1). Compared with N_{PL}, H_{PL} lowered pNN50, RMSSD, and HF at +10 h, whereas at +23 h, only pNN50 and RMSSD were decreased. KE did not alter the impact on RMSSD but lowered pNN50 and HF at +23 h compared with H_{PL}.

Resting Measurements—Acid–Base Balance, Blood Gasses, and Ventilatory Parameters

Blood pH, p50, pCO_2, as well as ventilatory parameters were similar between all conditions at baseline (Table 2). Compared with N_{PL}, H_{PL} increased blood pH at +10 and +23 h but not at +27 h, whereas pCO_2 was decreased at all time points. Conversely, p50 remained similar between N_{PL} and H_{PL} at all time points. Relative to H_{PL}, H_{KE} caused a slight acidosis at +10 h, which was accompanied by a KE-induced reduction in pCO_2 and an increase in p50. Each of these KE-induced alterations were again normalized at +23 and +27 h. Irrespective of the experimental condition, $\dot{V}E$ was similar at baseline (11.5 ± 0.2 $L \cdot min^{-1}$), increased at +10 h (13.1 ± 0.2 $L \cdot min^{-1}$, $P <$ 0.001 vs baseline), and decreased at +23 h (9.9 ± 0.4 $L \cdot min^{-1}$, $P = 0.001$ vs baseline). $\dot{V}O_2$ was similar at baseline (0.161 ± 0.014 $L \cdot min^{-1}$) and increased at +10 h (0.236 ± 0.009 $L \cdot min^{-1}$, $P = 0.004$ vs baseline). Also, $\dot{V}CO_2$ was similar at baseline (0.182 ± 0.012 $L \cdot min^{-1}$) yet decreased at +27 h (0.0125 ± 0.017 $L \cdot min^{-1}$, $P = 0.018$ vs baseline).

Nocturnal Catecholamine Excretion

Nocturnal urine production was similar in all conditions (N_{PL}: 679 ± 375 mL, H_{PL}: 626 ± 223 mL, H_{KE}: 685 ± 321 mL, $P = 0.647$). Also nocturnal adrenaline (N_{PL}: 5.86 ± 3.66 nmol, H_{PL}: 6.93 ± 4.16 nmol, H_{KE}: 6.60 ± 4.14 nmol, CV = 3.5%, $P = 0.305$), noradrenaline (N_{PL}: 52.5 ± 22.2 nmol, H_{PL}: 50.3 ± 10.8 nmol, H_{KE}: 56.7 ± 12.4 nmol, CV = 3.3%, $P = 0.608$), and dopamine (N_{PL}: 911 ± 305 nmol, H_{PL}: 795 ± 229 nmol, H_{KE}: 956 ± 350 nmol, CV = 3.9%, $P = 0.324$) excretions were unaffected by the different conditions.

TABLE 2. Effect of hypoxia (H_{PL} vs N_{PL}) and KE ingestion (H_{KE} vs H_{PL}) on blood acid base balance and capillary blood gases.

	N_{PL}	H_{PL}	H_{KE}
pH			
Baseline	7.409 ± 0.016	7.412 ± 0.013	7.406 ± 0.011
+10 h	7.419 ± 0.021	7.438 ± 0.017$^{\S,\#}$	7.395 ± 0.016$^{\S,\#,*}$
+13 h	7.414 ± 0.014	7.433 ± 0.018$^{\S,\#}$	7.412 ± 0.027
+23 h	7.421 ± 0.013$^{\#}$	7.451 ± 0.026$^{\S,\#}$	7.457 ± 0.009$^{\S,\#}$
+27 h	7.417 ± 0.015	7.431 ± 0.016$^{\#}$	7.432 ± 0.018$^{\#}$
p50 (mm Hg)			
Baseline	26.2 ± 0.5	25.9 ± 0.7	25.9 ± 0.9
+10 h	25.1 ± 1$^{\#}$	25.9 ± 0.7	26.8 ± 1.3§,*
+13 h	25.8 ± 0.9	25.8 ± 1	25.5 ± 1.4
+23 h	25.3 ± 1.3	25 ± 1	24.4 ± 1$^{\S,\#}$
+27 h	25.6 ± 1.1	25.7 ± 0.6	25.1 ± 1.2
pCO_2 (mm Hg)			
Baseline	41.2 ± 2.2	42 ± 1.8	41.8 ± 1.7
+10 h	42.4 ± 1.1	40.9 ± 1.4§	38 ± 1.2§,*
+13 h	42.4 ± 1.6	40 ± 2§	37.3 ± 2§,*
+23 h	41.5 ± 2.2	37 ± 2.1§	36.7 ± 1.5§
+27 h	42.3 ± 1.7	39.4 ± 1.6§	39 ± 1.9§

During a randomized, cross-over protocol, participants received either KE or placebo (PL) supplements and spent the night either at a simulated altitude of 3000 m (H_{PL} and H_{KE}) or in normoxia (N_{PL}). Participants' capillary blood was analyzed at baseline, after 2 h in hypoxia/normoxia (+10 h), immediately before sleep (+13 h), and upon waking up in hypoxia/normoxia (+23 h) and 2 h after returning to normoxia (+27 h). Capillary blood pH, p50, and pCO_2 were evaluated. $^{\S}P < 0.05$ versus N_{PL}; $^{*}P < 0.05$ versus H_{PL}; $^{\#}P < 0.05$ versus baseline.

Participant Blinding

After the final experimental session, participants indicated which supplement they thought to have received during each session and how confident they were of this answer scoring from 0% (not sure at all) to 100% (completely certain). Out of the 11 participants that were included in the data analysis, five participants were 0% to 50% sure (one out of five guessed correct) and six participants were 60% to 100% sure (two out of six guessed correct). No participants indicated to have noticed something else about the protocol or the altitude, indicating that the blinding of KE and hypoxia was successful.

DISCUSSION

In this research, we investigated whether KE ingestion could counteract sleep disruptions induced by sleeping at altitude (i.e., 3000 m) and impact next-day endurance exercise performance. Sleeping at altitude impaired sleep quality as evidenced by a 3% reduction in sleep efficiency, a doubling of WASO, and a ~20% reduction in SWS. These sleep disturbances did not affect performance during a 30-min time trial the next day but reduced subsequent HR recovery. KE slightly alleviated nocturnal hypoxemia at a single time point, but this was insufficient to negate these sleep disruptions, nor did it impact next-day exercise performance or subsequent HR recovery.

Although our results on WASO, SWS, and REM are in line with available literature, we observed that even lower simulated altitudes (i.e., 3000 m) can elicit a drop in sleep efficiency. As sleep efficiency depicts the time spent asleep over the time spent in bed, with the latter standardized over all sessions, this drop was established by a decreased time asleep through a doubling in WASO. The divergent versus convergent effects on SE and WASO, respectively, between our study and earlier studies likely resulted from the fact that we, in contrast to most of the earlier studies, standardized the total time in bed. This is supported by two earlier studies showing that under free-living conditions the observed increase in nocturnal wakefulness upon altitude is at least in part compensated by a ~5% increase in time in bed (9,10). Nevertheless, this increase in time in bed failed to reach statistical significance in both studies.

Increased awakenings upon hypoxia are generally attributed to a hypoxia-induced periodic breathing pattern (8,34). This phenomenon almost exclusively occurs during NREM sleep and especially during SWS, and causes short awakenings or arousals and disruption of SWS. Our observations indicating that hypoxia decreases SWS but not REM sleep, together with an increased amount of arousals, thus indirectly endorse the important role of periodic breathing in hypoxic sleep impairment. This is further supported by our observation that hypoxia

drastically increased the periodic variation of SpO_2 during the night, which is a central hallmark of periodic breathing (32).

Sleep disturbances can evoke a myriad of negative effects on processes implicated in exercise performance, training adaptation, and general health. This is evidenced by studies showing that large reductions in sleep quantity, i.e., reductions of 2 to 3 h a night for 1 to 5 d, for instance increase HR during submaximal exercise (17), suppress myofibrillar and sarcoplasmic protein synthesis rates (18,19), and impair mitochondrial respiratory function (19) and insulin sensitivity (20). Although some studies reported a concomitant drop in both sprint and endurance cycling performance (5,22), even severe sleep disruptions do not always evoke impairments in exercise performance (17,21). However, to our knowledge, no data is available on the impact of more subtle sleep disturbances such as those observed in our study.

The minor sleep disturbances induced by hypoxia in our study did not affect $TT_{30'}$ performance, nor altered resting HR, HRV, or respiratory parameters the day after. However, the disrupted night in hypoxia compromised HR recovery after $TT_{30'}$ as evidenced by the ~10 bpm higher HR in H_{PL} versus N_{PL} 5 min after completion of $TT_{30'}$. A reduced or delayed HR recovery reflects an impaired vagal activity (35), a strong predictor for morbidity (36), which is generally associated with cardiovascular disease (37) and overtraining (38). This impaired HR recovery upon hypoxia may be related to the hypoxia-induced reduction in SWS. Indeed, selective SWS suppression was previously found to disrupt sympathovagal balance by decreasing vagal tone (39) and stimulation of slow waves during sleep improved next-day cardiac function, as evidenced through an improved left-ventricular systolic function (40,41). This indicates that although exercise performance was not affected after one disrupted night, more subtle changes are present, which may in the longer term have implications for exercise recovery, performance, as well as cardiovascular or general health. However, more research is required to comprehend the scope and validity of this hypothesis.

In contrast to our hypothesis, KE ingestion did not counteract the hypoxia-induced sleep dysregulations. This conflicts with our earlier data showing that KE intake negated the sleep disruptions induced by strenuous late evening exercise under normoxic conditions (27). A potential explanation for these contrasting observations is the disparate effects of strenuous late evening exercise versus hypoxia on sleep architecture. Both conditions caused a decrease in sleep efficiency and an increase in WASO. However, although strenuous late evening exercise decreased REM sleep without affecting NREM sleep, hypoxia did not affect REM sleep but decreased SWS. This suggests that ketosis may only beneficially impact sleep when REM is disrupted. Despite the consequential physiological differences, this is supported by an earlier study showing that a ketogenic diet improved REM sleep in children with epilepsy (42), a condition typically associated with reduced REM sleep (43). A previously postulated hypothesis suggests that the ability of KE to counteract reductions in REM sleep was mediated through an increased dopamine signaling after KE ingestion (44,45), as dopamine plays an important role in the transition from NREM to REM sleep (46). However, in contrast to our earlier studies, KE intake did not affect nocturnal dopamine excretion in the current study.

An important factor in compromised hypoxic sleep is hypoxemia and subsequent periodic breathing. As described previously, periodic breathing induces periods of hypopnea and apnea, causing short awakenings especially during SWS (15). As minimum SpO_2 values are closely related to the apneic part of a periodic breathing cycle (47,48), the observed KE-induced increase (~+3%) in minimum SpO_2 and SpO_2 at 40% of sleep duration together with the diminished variance in nocturnal SpO_2 after 2 h of sleep suggests a slightly tempered periodic breathing behavior after KE ingestion. However, this effect was likely too small to translate in sleep improvements. As nocturnal respiration was not recorded in the current study, further research is required to identify if KE may impact periodic breathing.

On the other hand, hypoxemia activates a wide array of adaptive responses, among others establishing the respiratory alkalosis observed in H_{PL}, causing a left shift of the oxyhemoglobin dissociation curve (ODC) and thus favoring a higher SpO_2 for a given pO_2 (26). Ingestion of KE, contrarily, established a relative metabolic acidosis in agreement with earlier data (25,26), thereby pushing a right-shift of the ODC as evidenced by the observed increase in pO_2 at 50% SpO_2 (i.e., p50) (49). Nevertheless, average nocturnal SpO_2 values were similar after ingestion of KE compared with PL, and only after 40% of sleep duration, SpO_2 was higher in KE. This can at least partly be explained by the higher capillary pO_2 values that were observed immediately before sleep, i.e., after 5 h in hypoxia, in H_{KE} (56.8 ± 3.4 mm Hg) versus H_{PL} (53.3 ± 4.0 mm Hg), thereby compensating the right shift of the ODC. It should however be highlighted that pO_2 values were obtained using a capillary sampling method, which might underestimate actual arterial pO_2 (50).

As indicated above, KE increased SpO_2 at 40% of the night, as well as minimum nocturnal SpO_2, but did not impact SpO_2 at any other time point. This is most likely related either to the extent of hypoxemia. This is supported by our two earlier studies showing that KE attenuated hypoxemia only whenever SpO_2 values were below ~82% (25,26). For instance, at +10 h, participants were in ketosis but SpO_2 values had only dropped to ~90%. However, these requirements were fulfilled at 40% of the night, but not at the other time points where we measured SpO_2. Furthermore, during the final part of the night, participants were most likely no longer in ketosis (27). Although higher degrees of hypoxemia might provoke more pronounced effects, the limited effect of KE on oxygenation in our study remains to be interpreted with caution. The observation that this beneficial effect on SpO_2 only occurs when saturation drops below a certain cut-off value could be explained by the sigmoidal nature of the ODC in combination with the ketosis-induced right shift and increase in pO_2. Indeed, a SpO_2 of ~85% is located in the steep part of the ODC under "normal" physiological conditions. And as such, a small change in pO_2 will have a large impact on SpO_2. Conversely, at the right-hand side of the ODC, a change in pO_2 will only marginally affect

SpO_2. Besides hypoxemia, KB may also affect sleep by altering sympathetic nervous activity. In line with the literature (51,52), hypoxia caused an increase in both resting and nocturnal HR, as well as a decrease in all HRV parameters on the first evening of the protocol, thereby suggesting a relative dominance of the sympathetic nervous system. Upon waking up however, HF values had recovered but RMSSD and pNN50 values remained depressed. Although RMSSD and pNN50 are measures for overall HRV, HF isolates the high-frequency component (0.12 to 0.4 Hz) of the spectrum and entails information on ventilation and respiratory sinus arrhythmias, a phenomenon explaining fluctuation of HR and HRV together with natural breathing (53–55). As natural breathing is disrupted in hypoxia, both through periodic breathing and consistent hyperventilation, HF was indeed expected to increase (55). Although SWS is associated with low HRV in normoxia (56), sympathetic nerve activity is even higher during REM sleep compared with being awake (57). Although the exact relationship between sleep and sympathetic activity remains unclear, it cannot be ruled out that this increased sympathetic dominance is linked to the hypoxia-induced disruption of SWS. However, whether this is either a cause or a consequence remains to be identified.

Despite unaffected nocturnal adrenaline and noradrenaline concentrations, KE lowered HRV indices pNN50 and RMSSD upon waking up and increased nocturnal HR. This indicates that, in line with earlier studies performed both in normoxia (27,58,59) and hypoxia (25), KE ingestion further promoted sympathetic dominance. In this context, through manipulation of sympathetic activity, KB may only potentially benefit sleep quality when REM sleep, and not SWS, is disrupted.

Although this research offers novel insights, we want to acknowledge a few limitations. First, the human response to hypoxia is largely similar for normobaric versus hypobaric hypoxia (60,61). However, in the context of sleep, hypobaric hypoxia has been shown to more severely disrupt sleep (62,63). Second, only males were recruited for participation in this study design given that the variation in reproductive hormones has been shown to affect both REM and SWS duration, and consequently total sleep and sleep efficiency (64,65). Therefore, follow-up studies in female subjects are warranted. Third, caloric content of our KE supplement (~1960 kJ) was higher than our CON (~190 kJ) supplement. Our placebo contained fewer calories compared with our KE supplements, resulting in lower energy intake in the placebo versus KE conditions (~190 vs ~1960 kJ). Nevertheless, relative to the total energy intake during the protocol, this difference is rather marginal (~11%). Furthermore, all subjects received a caloric surplus, and therefore, we do not expect that this may have influenced our results. In addition, employing an isocaloric placebo containing medium-chain triglyceride oil, carbohydrates, or proteins would considerably alter substrate metabolism; therefore, we opted for a low-caloric placebo. Finally, we recognize that including an additional N_{KE} session may have provided additional insight. However, based on our available data, KE may only improve sleep whenever sleep is dysregulated (45,66). Therefore, and similar to our earlier study (27), we decided to not include an N_{KE} session.

CONCLUSIONS

In conclusion, we observed that a single night of hypoxic exposure (~3000 m altitude) has a detrimental impact on sleep quality, through reduced sleep efficiency, SWS, and increased WASO. Although this did not impact next-day exercise performance, it increased postexercise HR, suggesting that sleeping at simulated altitude might have minor implications for athlete training management. Contrary to our expectations, ketone ingestion did not mitigate any of these effects. However, we confirmed earlier data showing that KE can alleviate hypoxemia whenever SpO_2 values drop below ~82%; however, such drastic SpO_2 drop occurred only at one time point during the night. Taken together with our earlier observations, ingestion of ketones as a strategy to improve sleep and recovery appears effective when REM sleep is disrupted (e.g., through late-evening exercise), whereas disruption of NREM sleep (through hypoxic exposure) remains unaffected.

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