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RESEARCH ARTICLE

Physiological Adaptations to Environmental Stressors and Challenging Conditions

The effects of 2 days of intermittent exogenous ketosis at high altitude on baroreflex sensitivity and ventilation under hypoxic and hypercapnic conditions

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Abstract

High-altitude (HA) exposure induces an integrated physiological response to mitigate hypoxemia. Exogenous ketosis at simulated HA was previously shown to accentuate sympathetic activation and attenuate pulse oxygen saturation (Sp_{O_2}) decreases through hyperventilation. The aim of this study was to extend these findings by investigating the effects of intermittent exogenous ketosis (IEK) across 2 days at terrestrial HA (3,375 m) on baroreflex sensitivity, heart rate variability, and hypoxic/hypercapnic ventilatory responses. Thirty-four healthy active adults completed neutral, hypoxic, and hypercapnic (0.03 $F_{I_{CO_2}}$) exposures, each comprising 6 min of seated rest, once at sea level (SL) and once after 2 days at HA. Across the 2 days, participants intermittently ingested either ketone monoester supplements (IEK) or placebo (PLA). During each exposure, blood pressure, ventilation, Sp_{O_2}, and end-tidal CO_2 pressure ($P_{ET_{CO_2}}$) were continuously recorded, and arterialized capillary blood gas content was measured in the final 30 s. Baroreflex sensitivity and time-domain metrics of heart rate variability were reduced at HA ($P = 0.006$–0.043) but unaffected by group ($P = 0.288$–0.525). However, ventilation at HA under all three conditions was significantly higher in IEK compared with PLA (all $P < 0.001$). In hypoxia, this induced a higher Sp_{O_2} ($P = 0.038$) and capillary O_2 pressure ($P = 0.003$). In hypercapnia, this induced a lower $P_{ET_{CO_2}}$ and capillary CO_2 tension (both $P < 0.001$). These results extend previous findings, suggesting that IEK enhances ventilation at terrestrial HA after 2 days of exposure, with this effect being independent from baroreflex sensitivity or heart rate variability changes.

NEW & NOTEWORTHY This study demonstrates that 2 days of intermittent exogenous ketosis at 3,375 m terrestrial altitude does not alter baroreflex sensitivity or heart rate variability but significantly increases pulmonary ventilation under neutral, hypoxic, and hypercapnic conditions, improving oxygenation and lowering carbon dioxide retention. These findings suggest that ketone supplementation may enhance ventilatory acclimatization to high altitude via metabolic acidosis-driven respiratory stimulation, offering a nonpharmacological alternative to typical interventions used to support acclimatization.

autonomic control; blood pressure; hypobaric hypoxia; ketone bodies; sympathetic nervous system

INTRODUCTION

At high altitude (HA), several integrated physiological adaptations occur in response to reduced oxygen availability (1). Among the key sensory systems involved are chemoreceptors, which modulate pulmonary ventilation ($\dot{V}_E$) (2), and baroreceptors, which help regulate blood pressure despite hypoxia-induced impairments (3). The interplay of these systems under acute and chronic hypoxic conditions is pivotal to the cardiovascular, respiratory, and autonomic responses underlying adaptation (4–6). Sympathoexcitation, often pronounced during acute and sustained HA exposure (7–9), is a hallmark of these responses. Appropriate autonomic control may contribute to early acclimatization success (10). In this context, heart rate variability serves as a fundamental parameter, reflecting the balance between parasympathetic and sympathetic branches of the autonomic nervous system, which is particularly sensitive to hypoxic stress (11, 12).

Exogenously increasing blood ketone bodies (e.g., ketosis) has previously been shown to increase survival time in mice exposed to severe environmental hypoxia (13), laying the

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foundations for recent human studies. In one such study, oral ketone ester supplementation was found to improve tolerance to simulated HA (4,000 m), likely mediated by improved blood and tissue oxygenation both at rest (14) and during exercise (15, 16) in hypoxia. Such effects have been attributed to higher $\dot{V}_E$ resulting from the metabolic acidosis with exogenous ketosis (14–16).

Exogenous ketosis has also been shown to accentuate the shift toward sympathetic nervous system dominance induced by simulated HA, as quantified using heart rate variability metrics after 4 h at 4,000 m (14) and after 13 h at 3,000 m (17). In the former study, although acute increases in systolic blood pressure (SBP) were observed under hypoxic conditions, exogenous ketosis did not influence resting blood pressure parameters (14). This aligns with large-scale evidence indicating no effect of ketone supplementation on resting blood pressure at sea level (18). However, vascular function improved after 2 wk of daily ketone ester supplementation in obese adults (19), and 3 wk of 6 days/wk ketone ester supplementation inhibited the sympathetic overdrive induced by simultaneous endurance training overload (20). Together, this evidence suggests baroreflex sensitivity as a potential mechanism through which HA-adaptation may be facilitated with intermittent exogenous ketosis (IEK). Given the interdependence of these systems and their susceptibility to HA-hypoxia, assessing baroreflex sensitivity alongside $\dot{V}_E$ and heart rate variability may clarify whether, and how, exogenous ketosis influences cardiorespiratory and vascular responses to hypoxic stress.

A common feature of the aforementioned studies investigating exogenous ketone ingestion in an HA context is the normobaric nature of their hypoxic exposures (e.g., simulated HA). This approach carries with it several benefits, not least the ability to implement many rigorous controls for a scientifically sound experimental design. However, such studies lack the ecological validity that conducting experiments at terrestrial HA are able to provide, which might—in large part—be attributed to the hypobaria (21). Indeed, barometric pressure has been demonstrated to influence ventilatory (22), although not necessarily baroreflex (23), responses to hypoxia. A second consideration is that the current literature has provided intriguing evidence as to the effects of IEK across relatively short periods (up to ~24 h) of hypoxic exposure. However, typical HA sojourns extend across several days, so investigations across more prolonged exposure durations would carry both novelty and practical relevance. From a mechanistic standpoint, the peripheral chemoreceptors are known to be particularly sensitive to oxygen, whereas the central chemoreflex is modulated by carbon dioxide levels (24), and both interact with baroreflex activation (25). As such, investigating the integrated physiological responses to hypercapnia may uncover mechanisms underpinning any potential effects of IEK.

The aim of this study was to investigate the effects of 2 days of IEK at 3,375 m on baroreflex sensitivity, $\dot{V}_E$, and heart rate variability, compared with placebo ingestion (PLA), relative to equivalent measurements without supplementation at sea level (SL). Based on existing evidence, IEK was hypothesized to accentuate HA-induced sympathetic dominance, further decreasing heart rate variability and also, therefore, baroreflex sensitivity. At HA, $\dot{V}_E$ was expected to be accentuated with IEK relative to PLA.

MATERIALS AND METHODS

Study Overview

This randomized, placebo-controlled, parallel-group study consisted of two experimental sessions, each involving neutral, hypoxic, and hypercapnic exposures. The first was conducted at SL (SL; 295 m, Ljubljana, Slovenia; ambient temperature, 26 ± 1°C and relative humidity, 55 ± 5%) and the second at terrestrial HA (HA; 3,375 m, Rifugio Torino, Italy; ambient temperature, 22 ± 2°C and relative humidity, 25 ± 4%). For HA, participants traveled from Ljubljana, Slovenia to Courmayeur, Italy (1,300 m) by car (~7 h), before ascending to 3,375 m via a 20-min cable car journey at ~1400 h. They then resided at 3,375 m for two nights before commencing testing on the second morning, having been intermittently ingesting either exogenous ketone ester supplements (IEK) or placebo (PLA). The time spent under HA conditions before testing was (means ± SD) 45.1 ± 0.7 h (range 43.9–47.2 h).

The broader project was preregistered at www.clinicaltrials.gov (NCT06097754) and supported by both the National Committee for Medical Ethics at the Republic of Slovenia Ministry of Health (No. 0120–524/2022/3) and the Aosta Hospital Ethical Committee (06/05/2021.0038781.I). All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants as part of the recruitment process.

Participants

A convenience sampling strategy was used, through which 41 participants were initially recruited via email outreach, poster advertisements, and personal referrals. Participants were eligible if aged between 18 and 35 yr, with a body mass index in the healthy range (18.5–25.0 kg/m^2), a permanent residence <1,000 m above sea level, and self-reporting as being regularly physically active. Exclusion criteria included altitude exposure >2,000 m within 1 mo before participation in either experimental session, recent or current adherence to a ketogenic diet and/or use of exogenous ketone supplements, habitual smoking status, and the presence of any chronic diseases or use of medications that could bias study outcomes or pose undue health risk. In line with these criteria, 36 of the initial 41 participants were retained. A complete blood count assessment was conducted at baseline to verify health status. Given that two participants withdrew between the SL and HA phases, 34 participants were included in the final analysis (n = 17 per group, n = 3 women per group). Participant characteristics for these individuals are presented in Table 1.

Supplementation Strategy

To establish a true baseline, no supplements were administered at SL. For HA, participants were randomly assigned to IEK or PLA, stratified by peak oxygen uptake based on an incremental exercise test to exhaustion conducted at baseline. Figure 1 presents a schematic overview of the study, including the dosing strategy and blood ketone concentration data relative to HA arrival and the HA testing session. A final supplement was ingested 30 min before the HA testing

Table 1. *Participant characteristics*

Characteristic	IEK (n = 17)	PLA (n = 17)
Age, yr	23 ± 2	24 ± 3
Body mass index, kg/m^2	22.8 ± 2.0	22.3 ± 1.9
Peak oxygen uptake rate, mL/kg/min	53.5 ± 7.0	53.7 ± 5.3
Peak power, W	326 ± 65	318 ± 45
Blood hemoglobin concentration, g/L	14.9 ± 1.7	15.0 ± 1.0
Hematocrit, %	43 ± 4	45 ± 3
Forced vital capacity, L	5.7 ± 1.0 (101 ± 12)	5.5 ± 0.8 (102 ± 10)
FEV_1, L	4.6 ± 0.8 (97 ± 14)	4.4 ± 0.7 (97 ± 11)
Peak expiratory flow rate, L/s	9.2 ± 2.2	9.7 ± 2.1

All values are means ± SD. Spirometry data presented in brackets are expressed as the percentage of the predicted values based on standard reference equations (26). Peak oxygen uptake rate and peak power were assessed during preliminary testing using a ramp-incremental exercise test (2 min @ 50 W then +1 W/3 s until volitional exhaustion) on a cycle ergometer. FEV_1, forced expiratory volume in 1 s; IEK, intermittent exogenous ketosis group; PLA, placebo group.

session commenced to ensure IEK participants would complete the test in a state of exogenously induced ketosis, which was verified using earlobe capillary blood D-β-hydroxybutyrate concentration measurements immediately before the recordings began (GlucoMen Areo 2K meter with β-ketone sensor strips, A. Menarini Diagnostics, Firenze, Italy) (means ± SD; IEK vs. PLA, 3.8 ± 0.7 vs. 0.4 ± 0.1 mM, $P <$ 0.001). These D-β-hydroxybutyrate concentration measurements were conducted by a researcher who was otherwise not involved in the data collection procedures.

On each supplementation occasion, IEK participants ingested 25 g of the ketone monoester (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (KetoneAid Inc., Falls Church). The PLA participants instead ingested similar boluses of an inert placebo, containing 33 g of water, with an additional 1 mM sucrose octaacetate (Sigma-Aldrich, Bornem, Belgium) to mimic the bitterness of the ketone solution. Eight percent vol/vol sucralose (MyProtein, New York City) was dissolved in water and added to all supplements to soften the bitter taste of the ketone and taste-matched placebo supplements. Eight drops of strawberry flavoring (MyProtein, New York City) were added to all supplements to prevent identification of the specific odor of the ketone supplements. All supplements were referred to as "ketones" regardless of group assignment and provided in nontransparent 50-mL tubes to prevent visual identification. Participants were required to thoroughly shake the tube, consume the full contents, and immediately flush the tube with 30–40 mL Coca-Cola Zero Sugar Zero Caffeine (The Coca-Cola Company, Atlanta) to ensure complete ingestion.

Supplements were prepared and administered by a researcher who was otherwise not involved in the testing procedures. The researchers responsible for recording the experimental measurements successfully remained unaware of group assignment. Participants were asked via exit questionnaire which group they believed they had been assigned to, and their confidence in their response was recorded [from 0% ("no idea at all") to 100% ("completely certain")]. In both groups, 11 of the 17 participants (65%) correctly guessed their group assignment, with similar confidence levels for IEK and PLA participants (67 ± 15 vs. 66 ± 16%).

Experimental Protocol

Participants abstained from caffeine (≥24 h), alcohol (≥24 h), and exercise (≥12 h) before each session. All tests in both locations were conducted in the late morning

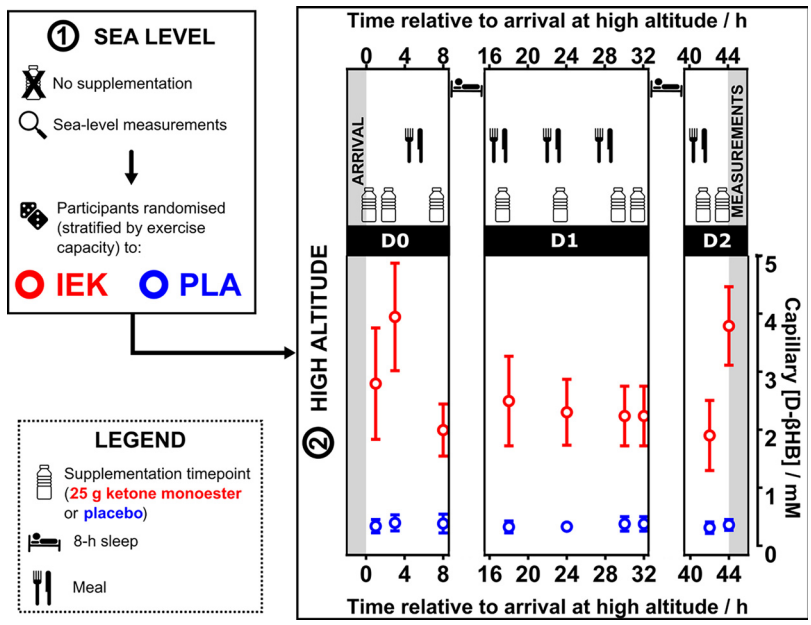

Figure 1. Schematic overview of the experimental design, including capillary D-β-hydroxybutyrate concentration (D-βHB) throughout the ~48 h high-altitude exposure in participants assigned to the intermittent exogenous ketosis (IEK; red) and placebo (PLA; blue) groups. Data represent means ± SD. Capillary blood measurements are always performed 30 min after supplement ingestion. The final capillary D-β-hydroxybutyrate concentration measurement coincides with the start of the high-altitude measurements.

(1000–1230 h), at a similar time within individuals (Median [IQR] ±23 [33] minutes). Participants were seated in a comfortable chair and maintained a state of quiet restfulness during calibration and instrumentation. A vasodilatory cream (Capsolin, SIT s.r.l., Mede, Italy) was applied to the right earlobe to locally arterialize capillary blood. Before each test, the gas sensors and turbine flow sensor from a metabolic cart (QUARK CPET, COSMED, Rome, Italy) were calibrated using a gas mixture (16% O_2; 5% CO_2) and a 3-L calibration syringe, respectively, as per manufacturer guidelines. A continuous noninvasive fingertip blood pressure monitor (NIBP100D, Biopac Systems Inc., Goleta) was calibrated for each participant by measuring brachial blood pressure using a standard occlusion cuff on the upper left arm. A double finger cuff was placed over the index and middle fingers of the right hand. Pressure was measured continuously at the fingertip and automatically scaled to the brachial calibration value. A pulse oximeter (Xpod 3012LP, Nonin Medical Inc., Plymouth) was placed on the left earlobe. A low dead-space facemask (7450 V2 series, Hans Rudolph, Kansas City) comfortably and securely covered the mouth and nose and was connected to a two-way nonrebreathing valve (2700 series, Hans Rudolph, Kansas City). Plastic flexible tubing was attached to the inhalation port of the valve, through which inspired gas combinations could be administered in a single-blinded manner. Participants were instructed to remain relaxed and breathe normally throughout the testing period. A three-way valve (2100 series, Hans Rudolph, Kansas City) was used to transition between isobaric gas reservoirs that were replenished ad hoc from compressed gas cylinders. The inspired gas composition for each exposure is reported in Table 2. The three exposures in each location are referred to as neutral (normoxic normocapnia), hypoxia (hypoxic normocapnia), and hypercapnia (normoxic hypercapnia). The fraction of inspired oxygen for the SL hypoxia (0.138) and HA neutral and hypercapnia (0.32) exposures was calculated a priori to match the partial pressure of inspired oxygen based on expected barometric pressure differences between locations. Each exposure lasted 6 min. In the final 30 s of each exposure, an arterialized capillary blood sample was collected from the right earlobe.

Data Acquisition

Blood pressure was sampled continuously at 1,000 Hz using an analog-to-digital converter (MP150, Biopac Systems Inc., Goleta), interfaced to a personal computer, and recorded using AcqKnowledge v. 4.2 software (Biopac Systems Inc., Goleta). Data were exported at 1,000 Hz for later analysis. $\dot{V}_E$ (L/min), Sp_{O_2} (%), and end-tidal partial pressure of carbon dioxide ($P_{ET_{CO_2}}$) were sampled and exported breath-by-breath using OMNIA v. 2.2 software (COSMED, Rome, Italy). Arterialized capillary blood samples were immediately analyzed using a blood gas analyzer (ABL90 FLEX PLUS, Radiometer, Copenhagen, Denmark) for determination of arterialized capillary oxygen pressure (Pa_{O_2}; mmHg), carbon dioxide pressure (Pa_{CO_2}; mmHg), bicarbonate ($[HCO_3^-]$; mM), pH, and the Pa_{O_2} at which hemoglobin is 50% saturated with oxygen (p50; mmHg).

Data Processing

Data were processed using Python v.3.11.9 (Python Software Foundation, Wilmington) in Visual Studio Code v. 1.97.2 (Microsoft, Redmond). All blood pressure and metabolic cart files from SL and HA were divided into their respective neutral, hypoxia, and hypercapnia exposures.

To allow cardiovascular stabilization, the first minute of blood pressure data within each exposure was excluded. The remaining data were low-pass filtered (4th-order Butterworth, 15 Hz cut-off) to reduce high-frequency noise while preserving systolic and diastolic peak fluctuations. Systolic blood pressure (SBP) peaks were identified algorithmically, with a minimum peak distance of 300 ms and a minimum peak height threshold dynamically set as the 75th percentile of the entire pressure signal within each exposure window. To refine peak locations, a second-order polynomial was fitted to five consecutive points centered around each identified peak, and the maximum point on the fitted curve was selected (27). All raw data files were then visually inspected in their entirety; erroneously identified peaks were omitted, and undetected SBP peaks were manually added using the aforementioned polynomial refinement technique. Inter-beat intervals (IBIs) were calculated as the time difference between successive SBP peaks. To minimize signal artifacts, only values deemed to be within a physiological range were retained (SBP: 40–200 mmHg, IBI: 0–2,000 ms). IBIs were verified by comparing the average heart rate in the final 60 s of each exposure with that recorded by the metabolic cart using earlobe pulse oximetry (overall mean bias: −0.23 beats/min, 95% limits of agreement: −1.60 to 1.07 beats/min). Diastolic blood pressure (DBP) troughs were identified algorithmically by identifying the minimum pressure value between each SBP peak, within the final third of the subsequent IBI, to avoid the dicrotic

Table 2. *Summary of the six environmental conditions under which blood pressure, pulmonary ventilation, pulse oxygen saturation, and arterialized capillary blood gases were measured*

Location (Altitude)	Barometric Pressure, mmHg	Condition	Composition of Inspired Gas, %		Measured Partial Pressure, mmHg	
			Oxygen	Carbon Dioxide	Oxygen	Carbon Dioxide
Sea level (295 m)	737 ± 4	Neutral	Ambient air		143.5 ± 1.0	0.9 ± 0.2
		Hypoxia	13.80	0.03	98.0 ± 2.7	0.6 ± 0.1
		Hypercapnia	20.94	3.00	148.0 ± 5.5	19.4 ± 0.9
High altitude (3,375 m)	509 ± 7	Hypoxia	Ambient air		95.8 ± 1.5	0.8 ± 0.1
		Neutral	32.00	0.03	148.8 ± 2.8	0.4 ± 0.1
		Hypercapnia	32.00	3.00	148.9 ± 2.4	13.2 ± 0.4

All values are means ± SD. Gas compositions were calculated a priori by considering the expected difference in barometric pressure between Ljubljana, Slovenia and Rifugio Torino, Italy. Composition of inspired gas (%) represents the value reported on the gas bottles during testing. Measured partial pressure values were calculated using the fraction of inspired gases and barometric pressure values recorded breath-by-breath by the metabolic cart in the final minute of each exposure.

notch. Mean arterial pressure (MAP) was then calculated as DBP + [(SBP − DBP)/3]. Mean SBP, DBP, MAP, and heart rate were then calculated from the final 60 s of each exposure.

Baroreflex sensitivity was quantified using the sequence method (28), following established validity criteria (27). Valid sequences were identified as those containing ≥3 consecutive heartbeats in which both SBP and IBI changed in the same direction and met thresholds of $|\Delta SBP| \geq 1$ mmHg and $|\Delta IBI| \geq 5$ ms. For each identified sequence, Pearson's correlation coefficient (r) was computed between ΔSBP and ΔIBI, and sequences were retained only if $r \geq 0.85$. A minimum of five valid sequences was required for an overall baroreflex sensitivity estimate to be considered valid. The slope of the linear regression between ΔSBP (independent variable) and ΔIBI (dependent variable) was computed for all retained sequences. Baroreflex sensitivity (ms/mmHg) under each condition was then quantified as a weighted arithmetic mean of all slope values, with the weighting determined by the number of data points underpinning each contributing linear regression slope value.

Heart rate variability was determined under the prevailing SL (normoxia) and HA (hypoxia) conditions by extracting the final 5 min of IBI data from the relevant (initial ambient) exposures. Ectopic beats were first identified as IBIs increasing by 32.5% or decreasing by 24.5% relative to the previous interval (29). These values were removed and compensated by linear interpolation. Given the influence of ectopic beats on measures of heart rate variability (30), recordings containing >5% ectopic beats were excluded from subsequent analysis ($n = 3$). The proportion of ectopic beats across the remaining recordings was median [IQR]: 0.00 [0.52]%. Conventional metrics of heart rate variability were then calculated (31). These included the root mean square of successive differences between adjacent IBIs (RMSSD, ms), the proportion of successive IBIs that differed by more than 50 ms (pNN50, %), and the low- (0.04–0.15 Hz, ms^2) and high-frequency (0.15–0.40 Hz, ms^2) signal components, together with the low-to-high frequency ratio, using Welch's spectral density estimate (Fast Fourier Transform; 4 Hz).

To establish ventilatory sensitivity, $\dot{V}E$, Sp_{O_2}, and PET_{CO_2} were quantified from the metabolic cart files by computing the arithmetic mean from the final minute of each exposure and are reported in absolute terms. The hypoxic (poikilocapnic) (HVR) and hypercapnic (normoxic) (HCVR) ventilatory responses were additionally quantified as $\Delta\dot{V}E/-\Delta Sp_{O_2}$ (L/min/%) and $\Delta\dot{V}E/\Delta PET_{CO_2}$ (L/min/mmHg), respectively, by comparing the relevant condition combinations in both locations (normoxic vs. hypoxic normocapnia and normocapnic vs. hypercapnic normoxia, respectively).

Sample Size Considerations

An a priori sample size estimation was conducted using G*Power (v. 3.1.9.8) (32). The present study was statistically powered to detect a moderate effect size (Cohen's $f = 0.25$) in baroreflex sensitivity, assuming a correlation among repeated measures of $r = 0.63$ [according to repeatability data from (33)]. With typically accepted type-I (α, 0.05) and type-II (β, 0.20) error rates, 26 participants (13 per group) were expected to be necessary. To account for potential dropout and/or data loss, 36 participants were initially included in baseline (SL) testing. Thirty-four participants were retained, with 17 participants randomly assigned to each group for the HA tests.

Due to the inherent challenges of conducting physiological research at HA and the stringent validity criteria for baroreflex sensitivity and heart rate variability outcomes in particular, certain data were unavailable or excluded from the final analysis. Contributing factors included participant fainting episodes, metabolic cart sensor malfunctions, capillary blood sample analysis errors, and cases where validity criteria during data processing were not met. A breakdown of the final sample size for each outcome measure is presented in Table 3.

Statistical Analysis

Statistical analyses were conducted using R v. 4.2.2 in RStudio v2024.09.1 + 394 (Posit PBC, Boston). All results are reported as means ± SD. Mixed effects models were defined for each outcome measure using the lmer function from the lmerTest package. Fixed effects were specified as group (IEK, PLA) and location (SL, HA), and a random intercept was included to account for within-participant correlations. Separate models were thus defined for each condition (e.g., neutral, hypoxia, and hypercapnia), considering that between-condition effects were not of primary interest in this study, and statistical power would be drastically reduced with the inclusion of a third (interacting) independent variable. In the case of a statistically significant interaction effect, pairwise comparisons were conducted using the emmeans package, with the resulting P values adjusted for multiple comparisons using the Ryan–Holm–Bonferroni stepwise adjustment. Statistical significance was accepted when $P < 0.05$.

RESULTS

HA Reduces Baroreflex Sensitivity and Increases Blood Pressure Independent of IEK

Baroreflex sensitivity was unaffected by group ($P = 0.288$–0.525) under all three conditions, and there were no group × location interaction effects ($P = 0.107$–0.134). Main effects of location (HA vs. SL) were, however, observed in hypoxic (8 ± 3 vs. 15 ± 8 ms/mmHg, $P = 0.032$, Fig. 2*A*), neutral (9 ± 3 vs. 17 ± 8 ms/mmHg, $P = 0.021$, Fig. 2*B*), and hypercapnic (9 ± 3 vs. 16 ± 7 ms/mmHg, $P = 0.039$, Fig. 2*C*) conditions.

No effects of group ($P = 0.448$–0.851) or interaction effects ($P = 0.303$–0.999) were detected for SBP. However, HA increased SBP relative to SL in all conditions (hypoxia, 140 ± 21 vs. 123 ± 17 mmHg, $P < 0.001$, Fig. 2*D*; neutral, 139 ± 17 vs. 124 ± 14 mmHg, $P = 0.007$, Fig. 2*E*; hypercapnia, 140 ± 19 vs. 125 ± 17 mmHg, $P = 0.014$, Fig. 2*F*). There was no main effect of group for heart rate under hypoxic conditions ($P = 0.382$), but heart rate was significantly higher at HA than SL (84 ± 13 vs. 76 ± 11 beats/min, $P < 0.001$, Fig. 2*G*). A significant interaction effect was observed in heart rate under both neutral ($P = 0.031$, Fig. 2*H*) and hypercapnic ($P = 0.028$, Fig. 2*I*) conditions. The interaction effects alluded to a greater increase in heart rate from SL to HA in IEK (neutral, 69 ± 10 vs. 83 ± 13 beats/min, $P < 0.001$; hypercapnia, 70 ± 9

Table 3. *Number of datapoints (participants) included in the final statistical models for each outcome measure*

Location (Altitude)	Group	HVR	HCVR	HRV	Condition	BRS	Pa_{O_2}	Pa_{CO_2}	$[HCO_3^-]$	pH	p50
Sea level (295 m)	IEK*	17	17	16	Neutral	12	16	16	16	16	16
					Hypoxia	13	15	17	13	17	13
					Hypercapnia	12	17	17	17	17	17
	PLA*	16	15	16	Neutral	15	17	17	17	17	17
					Hypoxia	14	15	15	15	15	15
					Hypercapnia	13	15	15	15	15	15
High altitude (3,375 m)	IEK	17	16	14	Neutral	15	17	17	17	17	17
					Hypoxia	11	17	17	16	17	15
					Hypercapnia	15	17	17	16	17	16
	PLA	16	15	16	Neutral	15	14	14	14	14	14
					Hypoxia	15	14	14	14	14	14
					Hypercapnia	15	14	14	13	14	13

All data represent a count of datapoints, out of the total possible sample size ($n = 17$). Outcome measures differentiated across location, group, and condition, as appropriate. Missing data attributed to participant fainting episodes, faulty metabolic cart sensors, BRS validity criteria not met (insufficient linear regression slopes), HRV validity criteria not met (>5% ectopic beats), and/or arterialized capillary blood sample analysis errors. BRS, baroreflex sensitivity; $[HCO_3^-]$, bicarbonate; HCVR, hypercapnic ventilatory response; HRV, heart rate variability; HVR, hypoxic ventilatory response; IEK, intermittent exogenous ketosis group; Pa_{CO_2}, arterialized capillary carbon dioxide partial pressure; Pa_{O_2}, arterialized capillary oxygen partial pressure; PLA, placebo group. *Note that no supplements were provided at sea level to establish a "true" baseline condition.

vs. 88 ± 13 beats/min, $P < 0.001$) compared with PLA (neutral, 69 ± 11 vs. 76 ± 11 beats/min, $P = 0.030$; hypercapnia, 69 ± 12 vs. 79 ± 10 beats/min, $P < 0.001$). However, no within-location between-group differences were observed between IEK and PLA at SL ($P = 0.857$ and $P = 0.747$, respectively) or at HA ($P = 0.167$ and $P = 0.054$, respectively).

At HA, DBP was increased compared with SL in neutral (84 ± 11 vs. 72 ± 9 mmHg, $P = 0.003$), hypoxic (85 ± 10 vs. 71 ± 9 mmHg, $P < 0.001$), and hypercapnic (84 ± 12 vs. 72 ± 10 mmHg, $P = 0.008$) conditions. However, there was no difference between IEK and PLA (neutral, 76 ± 11 vs. 79 ± 13 mmHg, $P = 0.270$; hypoxia, 77 ± 11 vs. 80 ± 13 mmHg, $P = 0.475$; hypercapnia, 77 ± 12 vs. 80 ± 13 mmHg, $P = 0.307$), and no interaction effect in any of the three conditions ($P = 0.749$, 0.787, 0.653, respectively). Similarly, MAP was increased at HA compared with SL in neutral (102 ± 11 vs. 89 ± 9 mmHg, $P < 0.001$), hypoxic (104 ± 12 vs. 89 ± 10 mmHg, $P < 0.001$), and hypercapnic (103 ± 13 vs. 90 ± 10 mmHg, $P = 0.004$) conditions, with no difference between IEK and PLA (neutral, 94 ± 10 vs. 97 ± 14 mmHg, $P = 0.250$; hypoxia, 95 ± 13 vs. 97 ± 14 mmHg, $P = 0.944$; hypercapnia, 95 ± 12 vs. 98 ± 14 mmHg, $P = 0.452$), and no interaction effects ($P = 0.701$, 0.483, 0.769, respectively).

HA Decreases Heart Rate Variability, with no Interacting Effects of IEK

No interaction effects were detected in any heart rate variability metrics ($P = 0.410$–0.644). Main effects of location were observed in RMSSD and pNN50, with a significant decrease from SL to HA noted in both (RMSSD: 62 ± 20 vs. 49 ± 21 ms, $P = 0.043$; pNN50: 38 ± 14 vs. 26 ± 13%, $P = 0.006$). No other main effects of location or group were observed in any metrics of heart rate variability ($P = 0.087$–0.839) (Table 4).

IEK at HA Accentuates Absolute $\dot{V}_E$, Decreasing $P_{ET_{CO_2}}$ and Mitigating Sp_{O_2} Decrements

No significant interaction effect ($P = 0.094$) or main effect of group ($P = 0.070$) was observed in the HVR. However, the HVR was higher at HA (0.406 ± 0.513 L/min/%) than at SL (0.079 ± 0.402 L/min/%; $P = 0.001$). There was also no significant interaction effect in the HCVR ($P = 0.065$). However, the HCVR was higher in IEK (1.343 ± 0.800 L/min/mmHg) than in PLA (0.905 ± 0.737 L/min/mmHg; $P = 0.002$) and higher at HA (1.513 ± 0.767 L/min/mmHg) than at SL (0.768 ± 0.640 L/min/mmHg; $P < 0.001$).

When assessed in absolute terms, $\dot{V}_E$ and PET_{CO_2} were similar between groups at SL under all three conditions ($P = 0.387$–0.973). At HA, $\dot{V}_E$ was higher in IEK than PLA in all conditions (hypoxia, 16.4 ± 2.7 vs. 12.8 ± 2.0 L/min, Fig. 3*A*; neutral, 14.5 ± 2.1 vs. 11.6 ± 1.9 L/min, Fig. 3*B*; hypercapnia, 20.6 ± 3.1 vs. 15.7 ± 2.6 L/min, Fig. 3*C*; all $P < 0.001$). Furthermore, IEK demonstrated lower PET_{CO_2} than PLA at HA in hypoxic (28 ± 3 vs. 33 ± 5 mmHg, $P = 0.002$, Fig. 3*D*), neutral (29 ± 4 vs. 34 ± 5 mmHg, $P < 0.001$, Fig. 3*E*), and hypercapnic (32 ± 3 vs. 38 ± 6 mmHg, $P < 0.001$, Fig. 3*F*) conditions.

A significant interaction effect was found for Sp_{O_2} in hypoxia ($P = 0.013$, Fig. 3*G*). There was no difference between groups at SL (IEK vs. PLA; 92.7 ± 2.2 vs. 93.0 ± 2.4%, $P = 0.592$), whereas Sp_{O_2} was higher in IEK compared with PLA at HA (95.6 ± 1.5 vs. 93.8 ± 2.3%, $P = 0.038$). No interaction effect was found under neutral conditions ($P = 0.292$, Fig. 3*H*), and there was no main effect of group (IEK vs. PLA; 99.7 ± 0.4 vs. 99.5 ± 0.7%, $P = 0.059$). However, Sp_{O_2} was higher at HA than at SL (99.8 ± 0.4 vs. 99.5 ± 0.6%, $P = 0.005$). No Sp_{O_2} interaction effect was found in hypercapnia ($P = 0.086$, Fig. 3*I*), and there was no main effect of location (HA vs. SL; 99.8 ± 0.4 vs. 99.9 ± 0.3%, $P = 0.353$), but Sp_{O_2} was higher in IEK than in PLA (99.9 ± 0.2 vs. 99.8 ± 0.5%, $P = 0.017$).

IEK Alters Acid-Base Balance and Blood Gas Content at HA, Indicating Enhanced Respiratory Compensation and Shifts in Oxygen Delivery

Significant group × location interaction effects were observed for several arterialized capillary blood variables under all conditions. Notably, $[HCO_3^-]$ was significantly lower in IEK than PLA at HA under neutral (16.8 ± 2.0 vs. 21.9 ± 0.8 mM), hypoxic (16.4 ± 2.1 vs. 21.6 ± 1.0 mM), and

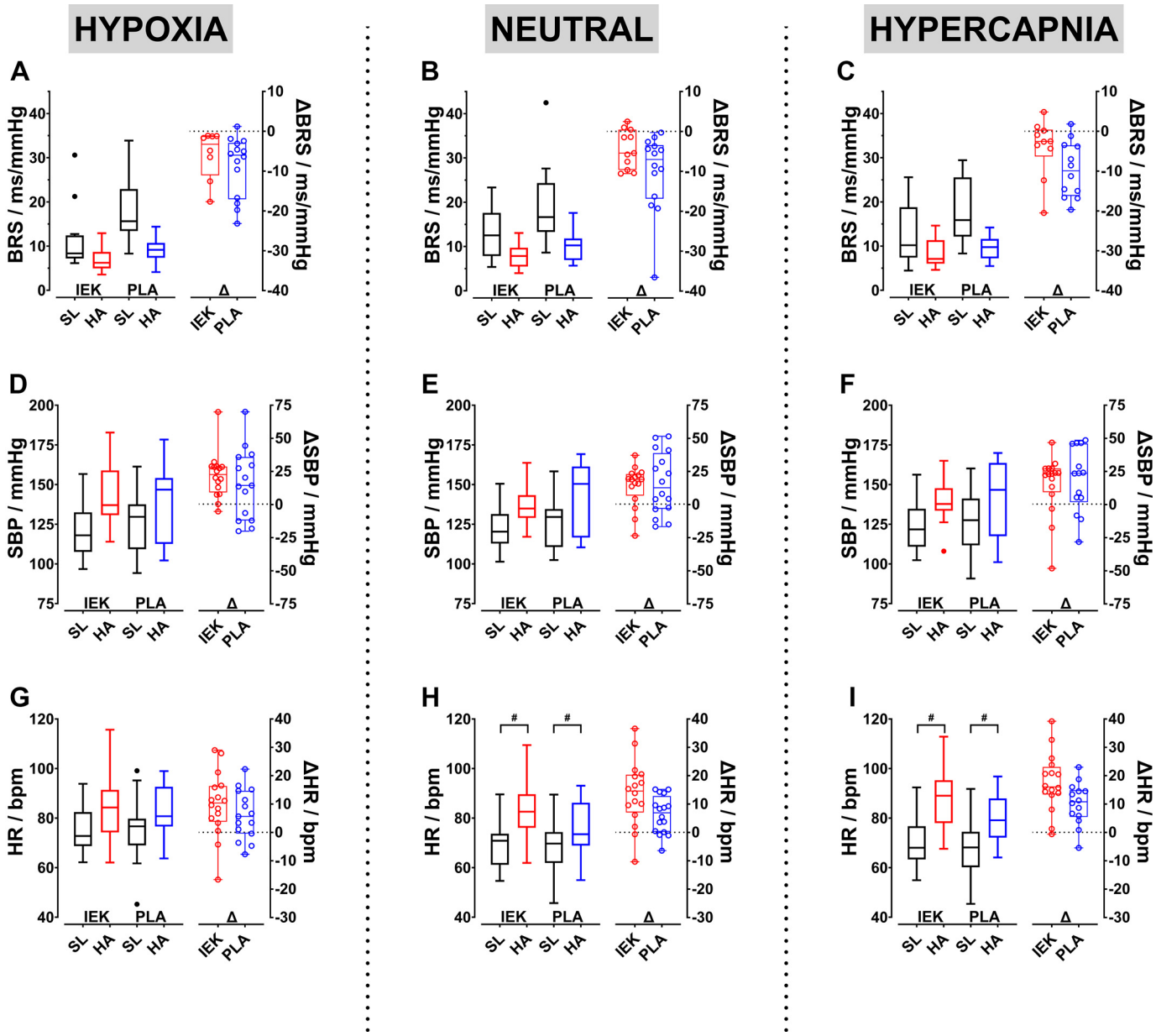

Figure 2. Baroreflex sensitivity (BRS; *A*–*C*), systolic blood pressure (SBP; *D*–*F*), and heart rate (HR; *G*–*I*) in participants assigned to the placebo (PLA) and intermittent exogenous ketosis (IEK) groups. Values measured without treatment at sea level (SL; black Tukey box plots), and at high altitude after 48 h of supplementation [HA; red (IEK) and blue (PLA) Tukey box plots]. Tukey box plots represent median, interquartile range, and range (within 1.5 times the interquartile range of the first and third quartiles). Absolute change scores (ΔHA-SL) from SL to HA are depicted using standard box and whisker plots superimposed by individual participant values. Measurements were conducted under hypoxic (*A*, *D*, and *G*), neutral (*B*, *E*, and *H*), and hypercapnic (*C*, *F*, and *I*) conditions in each location. Statistical inferences were derived from linear mixed models defined separately for neutral, hypoxia, and hypercapnia, each with location (HA vs. SL) and group (IEK vs. PLA) as fixed effects, and a random effect to account for repeated measures within participants. Significant interaction effects were further investigated using post hoc pairwise comparisons with Ryan–Holm–Bonferroni adjusted *P* values. $*P < 0.05$ for IEK vs. PLA (post hoc within-location pairwise comparison); $\#P < 0.05$ for HA vs. SL (post hoc within-group pairwise comparison).

hypercapnic (17.3 ± 2.0 vs. 22.4 ± 0.8 mM) conditions (all $P < 0.001$), with no between-group differences at SL ($P = 0.069$–0.875). Similarly, pH was significantly lower in IEK than PLA at HA under neutral (7.36 ± 0.02 vs. 7.42 ± 0.02), hypoxic (7.38 ± 0.02 vs. 7.44 ± 0.02), and hypercapnic (7.34 ± 0.02 vs. 7.41 ± 0.02) conditions (all $P < 0.001$), with no between-group differences at SL ($P = 0.069$–0.875). At HA, p50 was higher in IEK than PLA in neutral (27.2 ± 0.6 vs. 25.6 ± 0.8 mmHg, $P < 0.001$) and hypercapnic (27.7 ± 0.6 vs. 26.3 ± 0.8 mmHg, $P < 0.001$) conditions, with no difference at SL ($P = 0.830$ and 0.974, respectively). Under hypoxic conditions, there was no interaction effect ($P = 0.120$), but p50 was higher at HA than SL (25.9 ± 1.5 vs. 24.1 ± 1.1 mmHg, $P < 0.001$) and higher in the IEK than the PLA group (25.5 ± 1.7 vs. 24.6 ± 1.3 mmHg, $P = 0.012$). Blood gas results are shown in Fig. 4. Pa_{O_2} was significantly higher under hypoxic conditions in IEK than PLA at HA ($P = 0.003$), whereas no difference was noted between groups at SL ($P > 0.999$). Moreover, under hypercapnic

Table 4. *Results of heart rate variability analyses*

Metric	Sea Level		High Altitude		Statistical Inferences (*P* Values)		
	PLA	IEK	PLA	IEK	Interaction Effect	Main Effect of Location	Main Effect of Group
RMSSD, ms	68 ± 21	57 ± 17	54 ± 25	44 ± 14	0.410	**0.043**	0.308
pNN50, %	41 ± 14	35 ± 14	29 ± 12	23 ± 13	0.667	**0.006**	0.410
LF, ms^2	2,773 ± 3,146	1,080 ± 1,314	1,678 ± 1,932	635 ± 407	0.447	0.485	0.163
HF, ms^2	2,174 ± 1,720	1,469 ± 1,140	1,105 ± 864	732 ± 814	0.438	0.087	0.485
LF:HF Ratio	2.22 ± 2.42	1.25 ± 2.31	1.92 ± 2.17	1.43 ± 1.45	0.644	0.839	0.512

All values are means ± SD. Heart rate variability metrics were calculated using data from the final 5 min of each 6-min exposure, under the prevailing ambient conditions at sea level (normoxic normocapnia) and high altitude (hypoxic normocapnia). Mixed effects models were defined in which location and group were specified as fixed effects, and ID was specified as a random effect. Interaction effects represent location × group interactions. Statistically significant effects ($P < 0.05$) are highlighted in bold. HF, spectral power in the high frequency range (0.15–0.40 Hz); IEK, intermittent exogenous ketosis group; LF, spectral power in the low frequency range (0.04–0.15 Hz); PLA, placebo group; pNN50, proportion of successive inter-beat intervals differing by >50 ms; RMSSD, root mean square of successive differences.

conditions, IEK demonstrated lower Pa_{CO_2} than PLA at HA ($P < 0.001$), whereas at SL these values were comparable ($P = 0.373$).

DISCUSSION

The aim of this study was to investigate whether IEK across 2 days at 3,375 m altitude would affect baroreflex sensitivity, heart rate variability, and ventilatory responses under neutral, hypoxic, and hypercapnic conditions. The main results are:

1) Baroreflex sensitivity decreased at HA under all three conditions similarly in both groups.
2) Time-domain heart rate variability metrics indicated a shift toward sympathetic dominance at HA overall, but no differences were seen between groups. Frequency-domain heart rate variability metrics were affected by neither HA nor IEK.
3) IEK had a profound effect on $\dot{V}E$, with significantly greater hyperventilation at HA under all conditions relative to the control group. Such differences attenuated the Sp_{O_2} and Pa_{O_2} decrease in hypoxia at HA and attenuated the PET_{CO_2} and Pa_{CO_2} increases in hypercapnia at HA.

Baroreflex Sensitivity

In this study, 2 days of HA exposure at 3,375 m significantly decreased baroreflex sensitivity under all three conditions. This is in line with previous work where clear decreases were observed already upon acute (24 h) HA exposure (5,260 m), with further decreases noted after more prolonged (16-day) HA acclimatization (27). In another study, a group of healthy active male adults demonstrated reduced baroreflex sensitivity after ~20 h at 3,375 m relative to SL (34). In the present study, decreases induced by 2 days of hypobaric hypoxic exposure were detectable when comparing between equivalent levels of hypoxic, hypercapnic, and neutral (normoxic normocapnic) conditions, supporting the inference that prolonged HA exposure acted as the stimulus for such an effect, as opposed to the prevailing stimulus present at the time of measurement. The absolute values for baroreflex sensitivity were quantitatively similar to those previously observed in the same geographical locations (34), with the greater decrease in this study (~46 vs. ~34%) attributable to the additional 24 h of HA exposure. The mechanisms underlying these changes primarily involve shifts in autonomic nervous system activity, particularly sympathetic dominance in response to the physiological stress of HA-related hypoxemia, through which increased heart rate and blood pressure act together with alterations in baroreceptor function (35).

Although the HA reduction in baroreflex sensitivity was observed, IEK did not affect this response. In particular, no differences were found between groups at HA under any of the three conditions, conflicting with the a priori hypothesis regarding an accentuated shift toward sympathetic dominance with ketosis. This hypothesis had been generated through previous observations of autonomic nervous system alterations reflected in heart rate variability (14, 17), in line with the purported connection between these parameters (36). The fact that IEK did not affect baroreflex sensitivity or any heart rate variability parameters indicates that the shift in sympathetic dominance is either eradicated at the ~48 h timepoint or never existed at terrestrial HA despite those previous observations in normobaric hypoxia. From a methodological perspective, as can be seen in Fig. 2, *A–C*, the variability in baroreflex sensitivity measurements at SL was not particularly well distributed between the two groups. This may have precluded the identification of a clear interaction effect, although SBP, DBP, MAP, and heart rate were certainly better matched but also showed no potential IEK-specific effect.

Heart Rate Variability

As is to be expected upon exposure to HA (12), time-domain metrics of heart rate variability (RMSSD and pNN50) were reduced relative to SL. However, in line with the baroreflex sensitivity findings, no effects of IEK were noted. This appears to contradict previous work, in which exposure to normobaric hypoxia equivalent to ~4,000 m induced a greater attenuation in heart rate variability across the initial 4 h in participants who were in a state of exogenously induced ketosis, relative to when the same participants ingested a placebo on a separate occasion (14). In another study, after 2 h of normobaric hypoxic exposure (~3,000 m), an IEK protocol also lowered both pNN50 and RMSSD to a greater extent than hypoxia alone, compared with an appropriate placebo condition. In this regard, extrapolating to the ~48 h timepoint assessed in this study, it may be that heart

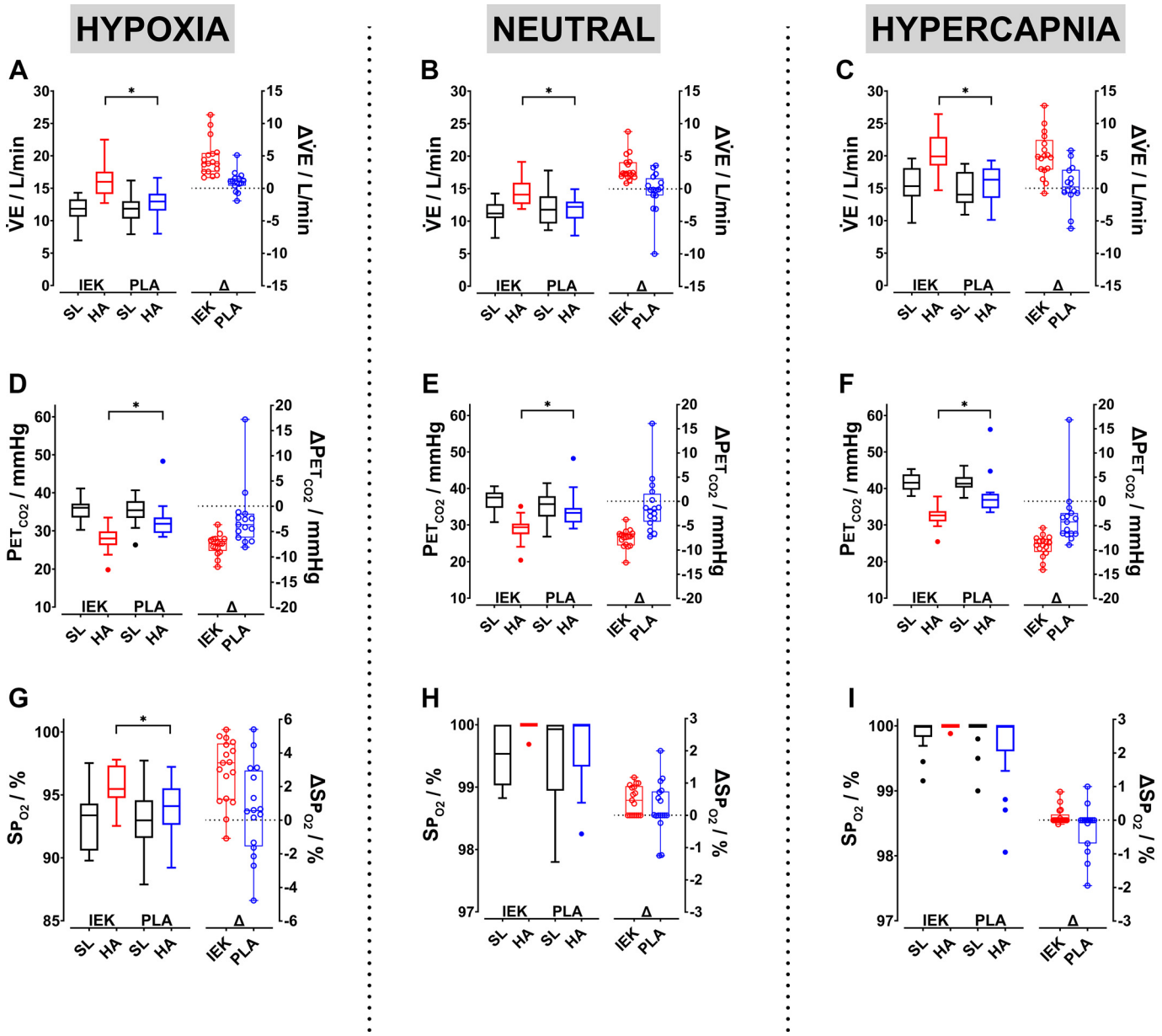

Figure 3. Pulmonary ventilation ($\dot{V}_E$; *A–C*), end-tidal partial pressure of carbon dioxide ($P_{ET_{CO_2}}$; *D–F*), and pulse oxygen saturation (Sp_{O_2}; *G–I*) in participants assigned to the placebo (PLA) and intermittent exogenous ketosis (IEK) groups. Values measured without treatment at sea level (SL; black Tukey box plots), and at high altitude after 48 h of supplementation [HA; red (IEK) and blue (PLA) Tukey box plots]. Tukey box plots represent median, interquartile range, and range (within 1.5 times the interquartile range of the first and third quartiles). Absolute change scores (ΔHA-SL) from SL to HA are depicted using standard box and whisker plots superimposed by individual participant values. Measurements were conducted under hypoxic (*A*, *D*, and *G*), neutral (*B*, *E*, and *H*), and hypercapnic (*C*, *F*, and *I*) conditions in each location. Statistical inferences were derived from linear mixed models defined separately for neutral, hypoxia, and hypercapnia, each with location (HA vs. SL) and group (IEK vs. PLA) as fixed effects, and a random effect to account for repeated measures within participants. Significant interaction effects were further investigated using post hoc pairwise comparisons with Ryan–Holm–Bonferroni adjusted *P* values. *$P < 0.05$ for IEK vs. PLA (post hoc within-location pairwise comparison).

rate variability differences induced by IEK at HA are transient (e.g., up to ~24 h). Alternatively, the sympathetic shift previously observed with IEK in hypoxia could be specific to simulated (normobaric) hypoxia. From another perspective, ketone supplementation during a chronic (3 wk) endurance training overload intervention attenuated the decrease in resting heart rate and suppressed nocturnal catecholamine excretion at the 1-, 2-, and 3-wk timepoints (20), suggesting a protective dampening of sympathetic activation in the context of chronic training stress. The context of the present study may therefore place it in between the sympathetic spike induced by acute (4 h) normobaric hypoxia (14) and the protective adaptation to more prolonged (1–3 wk) overtraining (20). Besides this potential time factor, it should also be noted that sympathetic excitation was not measured directly, for example, by quantifying muscle sympathetic nerve activity or through measurements of relevant biomarkers such as plasma norepinephrine. In any case, together

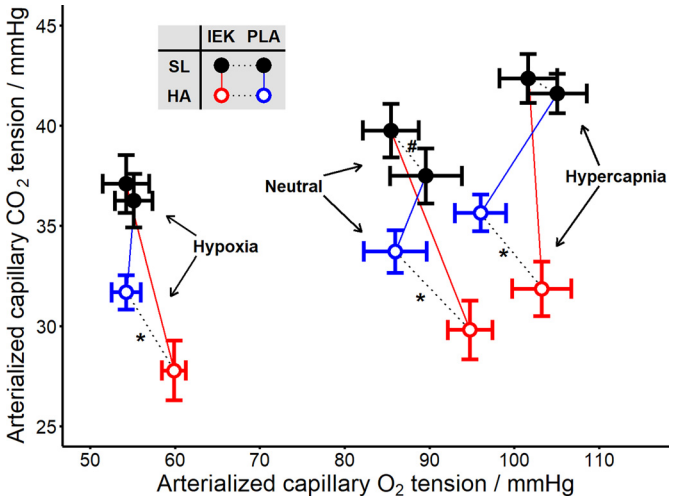

Figure 4. Arterialized capillary partial pressure of oxygen (O_2) plotted against arterialized capillary partial pressure of carbon dioxide (CO_2) in participants assigned to the placebo (PLA) and intermittent exogenous ketosis (IEK) groups. Values measured without treatment at sea level (SL; black circles) and at high altitude after 48 h of supplementation [HA; red (IEK) and blue (PLA) open circles]. Data represent means ± 95% confidence intervals. Measurements were conducted under neutral, hypoxic, and hypercapnic conditions in each location. Statistical inferences were derived from linear mixed models defined separately for neutral, hypoxia, and hypercapnia, each with location (HA vs. SL) and group (IEK vs. PLA) as fixed effects, and a random effect to account for repeated measures within participants. All interaction effects were statistically significant and were thus further investigated using post hoc pairwise comparisons with Ryan–Holm–Bonferroni adjusted *P* values. $*P < 0.05$ between groups for both O_2 and CO_2 (post hoc within-location pairwise comparison); $\#P < 0.05$ between groups for CO_2 only (post hoc within-location pairwise comparison).

with the baroreflex sensitivity data, these findings suggest no change in sympathetic drive with IEK after 2 days at terrestrial HA, acknowledging the indirect nature through which heart rate variability indicates sympathoexcitation.

Ventilatory Sensitivity

When quantified relative to ΔSp_{O_2} and $\Delta P_{ET}CO_2$, the poikilocapnic HVR and normoxic HCVR were both found to be higher at HA than at SL. This independent hypobaric effect, where $\dot{V}_E$ was only clearly increased in hypoxia compared with normoxia when at HA, was previously seen in a study of ours utilizing identical environmental stimuli (22). Regarding the primary comparison of interest in this study—the effects of IEK—no interaction effects were detected in the HVR or HCVR when quantified in this way. That said, the interaction effects in these two variables were $P = 0.094$ and $P = 0.065$, respectively, and since the present study was not statistically powered to detect these differences as the primary outcome, there may have been too few participants to reach statistical significance. Indeed, when the absolute values of $\dot{V}_E$, Sp_{O_2}, and $P_{ET}CO_2$ were compared, together with Pa_{O_2} and Pa_{CO_2} under each condition, IEK appeared to have induced profound ventilatory effects under all three conditions.

At SL, there were no differences between the two groups in any of these individual parameters, apart from a significantly higher Pa_{CO_2} under neutral conditions in the group subsequently assigned to IEK. Since no supplements were ingested at SL, we consider the baseline neutral Pa_{CO_2} difference to simply be a result of randomness in sampling and group assignment. At HA, where the participants had supplemented either ketone monoester or placebo across the preceding 2 days, the IEK group demonstrated significantly higher $\dot{V}_E$ under all three conditions than the PLA group (all $P < 0.001$). Under hypoxic conditions, this translated to significantly higher Sp_{O_2} and Pa_{O_2} in the IEK group compared with PLA. Similarly, under hypercapnic conditions, this translated to significantly lower $P_{ET}CO_2$ and Pa_{CO_2} in the IEK group compared with PLA. On average across the three conditions, the PLA group demonstrated a $\dot{V}_E$ of 13.4 ± 2.7 L/min, whereas $\dot{V}_E$ in the IEK group was 17.2 ± 3.6 L/min, a ~28% difference relative to PLA. Interestingly, these between-group differences are very similar to a previous study in which low-dose acetazolamide ingestion before a terrestrial altitude sojourn at 3,480 m was compared with a placebo condition across 3 days (37). Acetazolamide is a pharmacological aid often used in the treatment (or prevention) of acute mountain sickness (AMS), with accentuated $\dot{V}_E$ considered a primary physiological effect through which acclimatization responses are enhanced (38). In the aforementioned study, on *day 2* of the terrestrial altitude exposure, $\dot{V}_E$ in the placebo and acetazolamide groups was 14.6 ± 2.5 and 17.5 ± 3.3 L/min, respectively (37), remarkably similar to the effect observed with IEK in this study. Moreover, Pa_{O_2} was measured at 40.5 ± 3.4 and 48.2 ± 4.9 mmHg in the placebo and acetazolamide groups, respectively, although no differences were observed between their two groups in Sp_{O_2}. Importantly, although acetazolamide represents an effective pharmacological aid for altitude acclimatization, it has been found to impair cognitive function (39) and exercise capacity (40) in an HA context. Exogenous ketone ingestion, on the contrary, may facilitate cognitive performance in hypoxia (41) and seems not to impair exercise capabilities (15).

Previous research has demonstrated accentuated $\dot{V}_E$ in hypoxia with exogenously induced ketosis (14, 15), with similar effects also observed during exercise in normoxia (42) and hypoxia (16). The consistency of these findings—now extended to terrestrial HA after 48 h of exposure—warrants closer examination of the underlying mechanisms driving enhanced respiratory output. Ketone ester ingestion is known to increase the release of protons (43), supported in this study and others (14) by the consistently reduced pH, specifically with IEK at HA. Metabolic acidosis is in part compensated by the bicarbonate buffering system, with consistent [HCO_3^-] reductions also observed at HA under all three conditions. The central chemoreceptors are sensitive to such changes in pH and Pa_{CO_2} (24), and it may be that a state of exogenous ketosis facilitates a higher ventilatory rate to compensate for the altered acid-base balance. Metabolic acidosis is also known to induce a rightward shift of the oxyhemoglobin dissociation curve, enhancing oxygen unloading at a lower partial pressure in line with the principles of the Bohr effect (44). This was observed in the present study considering the higher p50 under neutral and hypercapnic conditions in the IEK group at HA. Although this would theoretically reduce Sp_{O_2} for a given Pa_{O_2}, the hyperventilation-induced increase in Pa_{O_2} with IEK compensated, ultimately increasing Sp_{O_2} despite the altered oxygen affinity (16).

Interestingly, such mechanisms parallel the aforementioned pharmacological aid, acetazolamide, which also induces a state of metabolic acidosis, albeit via a decrease in kidney bicarbonate reabsorption (38), as opposed to bicarbonate buffering. In either case, this metabolic acidosis directly stimulates chemoreceptor activity through modulated acid-base balance, and it may therefore be that exogenous ketosis represents a nonpharmacological approach to induce similar acclimatization-related physiological responses to those expected with acetazolamide. Although the present data primarily support an acid-base-mediated (indirect) effect of ketosis, it is also possible that ketone bodies modulate ventilation more directly. For example, β-hydroxybutyrate and acetoacetate have been shown to alter neuronal excitability through modulation of potassium ATP-sensitive channels (45) or neuronal potassium channels (46). Additional direct effects may include modulation of neurotransmitter systems and mitochondrial function, as well as other mechanisms influencing neuronal excitability (47).

Practical Considerations

Previous evidence demonstrated that exogenously induced ketosis at simulated HA (4,000–4,500 m) improved altitude tolerance and reduced AMS incidence after 4 h of exposure (14). Given that incidence at such altitudes is ∼50% and that incidence and severity peak after ∼6 h (48), such conditions were optimal to scrutinize the effects of ketosis. Despite these findings, AMS was not assessed in this study. Indeed, at a terrestrial altitude of 3,375 m, at the same location at which the present study was conducted, an incidence of 31% after 6 h and 15% after 14 h was noted in one study (49), and 12% after 16 h in another (34). As such, extrapolating to the 48 h timepoint would suggest that AMS incidence would be virtually 0% regardless of group assignment, and subjective participant observation suggested that this was indeed the case. Future studies may consider higher altitudes to determine whether the ventilatory effects observed here could translate into meaningful reductions in AMS severity and incidence.

Methodological Considerations

Several key methodological factors warrant discussion in relation to how the results may be interpreted. First, there was no specific dietary control in place during the HA exposure for logistical reasons. This may have influenced our results, since substrate availability is known to modestly affect ketone metabolism, even under resting conditions (50). That said, in our study, participants were provided with three regular meals per day at relatively consistent mealtimes, as dictated by the mountain hut. The random assignment of participants to each group is therefore likely to have distributed energy intake relatively evenly, and this is (to some extent) substantiated by body mass being virtually identical and unchanged between groups on both the first (IEK vs. PLA) (72 ± 10 vs. 69 ± 9 kg) and second (72 ± 10 vs. 70 ± 9 kg) mornings of the HA exposure. No evidence therefore exists for a substantive difference in caloric intake between groups, even despite the differences between supplements in caloric content (∼120 vs. 0 kcal/supplement). A second key factor relevant to the present study design is the potential for a period-by-treatment interaction. That is, the fact that all participants completed the SL before the HA condition means that it is not possible for the effects of location to be isolated from trial order effects. Importantly, trial order in this study was dictated by mountain hut availability, so trial order randomization was not possible. Moreover, ∼48 h of HA exposure may induce carryover effects, so such an approach was well justified. Besides, the statistically significant interaction effects (ventilatory outcomes) obtained in this study were scrutinized using between-group within-location differences, in addition to within-group between-location differences (e.g., "pre-to-post"-type comparisons), which supports our conclusions that group assignment (rather than trial order) explained the observed results.

Another key factor relates to sample characteristics. Despite open recruitment processes in relation to biological sex, only six (18%) of the study volunteers were women, and menstrual cycle status was subsequently not standardized for these individuals. Although this may limit generalizability, recent data suggest that the menstrual cycle does not significantly affect some of the HA-related physiological responses assessed in this study (49, 51). Nevertheless, further investigations regarding potential sex-related differences within the separate and interacting effects of ketosis and HA exposure seem warranted. Finally, a key aspect of the study was the intermittent nature of the supplementation protocol across 48 h. An additional supplement was consumed 30 min before HA testing to ensure a state of acute exogenous ketosis in the IEK group. This design means that the effect of the prolonged IEK protocol may not be separated from the acute state of exogenous ketosis at the time of testing. Considering that exogenous ketone supplementation had not yet been investigated at terrestrial HA across such a prolonged time period, the rationale was simply to prioritize identifying an effect, whether due to acute or cumulative ketosis. Having observed particularly these $\dot{V}_E$-related effects with such a design, a follow-up study that specifically separates the acute and cumulative effects is certainly warranted. Such a study, together with an investigation of the potential time course of the observed ventilatory effects, would help to refine potential guidelines.

Conclusions

In conclusion, although 2 days of HA exposure at 3,375 m significantly reduced baroreflex sensitivity and shifted autonomic activity toward sympathetic dominance, exogenous ketone ester supplementation did not modulate these responses. However, IEK led to substantial increases in $\dot{V}_E$, with notably higher values attenuating Sp_{O_2}/Pa_{O_2} reductions, and decreasing $P_{ET}CO_2/Pa_{CO_2}$, under respective hypoxic and hypercapnic conditions, relative to a control group. Together, these findings suggest that IEK across 2 days at HA may act to drive up $\dot{V}_E$ during acclimatization, but that these effects are independent from autonomic modulation. Further investigation into the feasibility and holistic effectiveness of IEK-based protocols for HA acclimatization is warranted.

DATA AVAILABILITY

The anonymized data are available via the Zenodo online data repository: https://doi.org/10.5281/zenodo.15427392. The

corresponding author may be contacted for any questions regarding the data or any other content relating to the manuscript.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

REFERENCES

1. **Wyatt FB.** Physiological responses to altitude: a brief review. *J Exerc Physiol Online* 17: 90–96, 2014.
2. **Duffin J.** Measuring the ventilatory response to hypoxia. *J Physiol* 584: 285–293, 2007. doi:10.1113/jphysiol.2007.138883.
3. **Beltrán AR**, **Arce-Álvarez A**, **Ramirez-Campillo R**, **Vásquez-Muñoz M**, **von Igel M**, **Ramírez MA**, **Del Rio R**, **Andrade DC.** Baroreflex modulation during acute high-altitude exposure in rats. *Front Physiol* 11: 1049, 2020. doi:10.3389/fphys.2020.01049.
4. **Marshall JM.** Chemoreceptors and cardiovascular control in acute and chronic systemic hypoxia. *Braz J Med Biol Res* 31: 863–888, 1998. doi:10.1590/s0100-879x1998000700002.
5. **Simpson LL**, **Stembridge M**, **Siebenmann C**, **Moore JP**, **Lawley JS.** Mechanisms underpinning sympathoexcitation in hypoxia. *J Physiol* 602: 5485–5503, 2024. doi:10.1113/JP284579.
6. **Alvarez-Araos P**, **Jiménez S**, **Salazar-Ardiles C**, **Núñez-Espinosa C**, **Paez V**, **Rodriguez-Fernandez M**, **Raberin A**, **Millet GP**, **Iturriaga R**, **Andrade DC.** Baroreflex and chemoreflex interaction in high-altitude exposure: possible role on exercise performance. *Front Physiol* 15: 1422927, 2024. doi:10.3389/fphys.2024.1422927.
7. **Berthelsen LF**, **Fraser GM**, **Simpson LL**, **Vanden Berg ER**, **Busch SA**, **Steele AR**, **Meah VL**, **Lawley JS**, **Figueroa-Mujíca RJ**, **Vizcardo-Galindo G**, **Villafuerte F**, **Gasho C**, **Willie CK**, **Tymko MM**, **Ainslie PN**, **Stembridge M**, **Moore JP**, **Steinback CD.** Highs and lows of sympathetic neurocardiovascular transduction: influence of altitude acclimatization and adaptation. *Am J Physiol Heart Circ Physiol* 319: H1240–H1252, 2020. doi:10.1152/ajpheart.00364.2020.
8. **Lundby C**, **Calbet J**, **van Hall G**, **Saltin B**, **Sander M.** Sustained sympathetic activity in altitude acclimatizing lowlanders and high-altitude natives. *Scand J Med Sci Sports* 28: 854–861, 2018. doi:10.1111/sms.12976.
9. **Simpson LL**, **Busch SA**, **Oliver SJ**, **Ainslie PN**, **Stembridge M**, **Steinback CD**, **Moore JP.** Baroreflex control of sympathetic vasomotor activity and resting arterial pressure at high altitude: insight from Lowlanders and Sherpa. *J Physiol* 597: 2379–2390, 2019. doi:10.1113/JP277663.
10. **Berger MM**, **Sareban M**, **Bärtsch P.** Acute mountain sickness: do different time courses point to different pathophysiological mechanisms? *J Appl Physiol (1985)* 128: 952–959, 2020. doi:10.1152/japplphysiol.00305.2019.
11. **Tanner V**, **Faiss R**, **Saugy J**, **Bourdillon N**, **Schmitt L**, **Millet GP.** Similar supine heart rate variability changes during 24-h exposure to normobaric vs. hypobaric hypoxia. *Front Neurosci* 15: 777800, 2021. doi:10.3389/fnins.2021.777800.
12. **Aebi MR**, **Bourdillon N**, **Bron D**, **Millet GP.** Minimal influence of hypobaria on heart rate variability in hypoxia and normoxia. *Front Physiol* 11: 1072, 2020. doi:10.3389/fphys.2020.01072.
13. **Kirsch JR**, **D'Alecy LG**, **Mongroo PB.** Butanediol induced ketosis increases tolerance to hypoxia in the mouse. *Stroke* 11: 506–513, 1980. doi:10.1161/01.str.11.5.506.
14. **Stalmans M**, **Tominec D**, **Lauriks W**, **Robberechts R**, **Debevec T**, **Poffé C.** Exogenous ketosis attenuates acute mountain sickness and mitigates normobaric high-altitude hypoxemia. *J Appl Physiol (1985)* 137: 1301–1312, 2024. doi:10.1152/japplphysiol.00190.2024.
15. **Poffé C**, **Robberechts R**, **Podlogar T**, **Kusters M**, **Debevec T**, **Hespel P.** Exogenous ketosis increases blood and muscle oxygenation but not performance during exercise in hypoxia. *Am J Physiol Regul Integr Comp Physiol* 321: R844–R857, 2021. doi:10.1152/ajpregu.00198.2021.
16. **Stalmans M**, **Tominec D**, **Lauriks W**, **Robberechts R**, **Ramaekers M**, **Debevec T**, **Poffé C.** Ketone ester ingestion impairs exercise performance without impacting cognitive function or circulating EPO during acute hypoxic exposure. *J Appl Physiol (1985)* 138: 1309–1320, 2025. doi:10.1152/japplphysiol.00097.2025.
17. **Stalmans M**, **Tominec D**, **Robberechts R**, **Lauriks W**, **Ramaekers M**, **Debevec T**, **Poffé C.** A single night in hypoxia either with or without ketone ester ingestion reduces sleep quality without impacting next day exercise performance. *Med Sci Sports Exerc* 57: 807–819, 2025. doi:10.1249/MSS.0000000000003604.
18. **Marcotte-Chénard A. Tremblay R**, **Falkenhain K**, **Little JP**, **Riesco E.** Effect of acute and chronic ingestion of exogenous ketone supplements on blood pressure: a systematic review and meta-analysis. *J Diet Suppl* 21: 408–426, 2024. doi:10.1080/19390211.2023.2289961.
19. **Walsh JJ**, **Neudorf H**, **Little JP.** 14-Day ketone supplementation lowers glucose and improves vascular function in obesity: a randomized crossover trial. *J Clin Endocrinol Metab* 106: e1738–e1754, 2021. doi:10.1210/clinem/dgaa925.
20. **Poffé C**, **Ramaekers M**, **Van Thienen R**, **Hespel P.** Ketone ester supplementation blunts overreaching symptoms during endurance training overload. *J Physiol* 597: 3009–3027, 2019. doi:10.1113/JP277831.
21. **Millet GP**, **Debevec T.** CrossTalk proposal: barometric pressure, independent of PO2, is the forgotten parameter in altitude physiology and mountain medicine. *J Physiol* 598: 893–896, 2020. doi:10.1113/JP278673.
22. **Narang BJ**, **Manferdelli G**, **Bourdillon N**, **Millet GP**, **Debevec T.** Ventilatory responses to independent and combined hypoxia, hypercapnia and hypobaria in healthy pre-term-born adults. *J Physiol* 602: 5943–5958, 2024. doi:10.1113/JP285300.
23. **Bourdillon N**, **Saugy J**, **Schmitt L**, **Rupp T**, **Yazdani S**, **Vesin J-M**, **Millet GP.** Acute and chronic changes in baroreflex sensitivity in hypobaric vs. normobaric hypoxia. *Eur J Appl Physiol* 117: 2401–2407, 2017. doi:10.1007/s00421-017-3726-6.
24. **Duffin J.** Measuring the respiratory chemoreflexes in humans. *Respir Physiol Neurobiol* 177: 71–79, 2011. doi:10.1016/j.resp.2011.04.009.
25. **Cooper VL**, **Pearson SB**, **Bowker CM**, **Elliott MW**, **Hainsworth R.** Interaction of chemoreceptor and baroreceptor reflexes by hypoxia and hypercapnia – a mechanism for promoting hypertension in obstructive sleep apnoea. *J Physiol* 568: 677–687, 2005. doi:10.1113/jphysiol.2005.094151.
26. **Bourdillon N**, **Yazdani S**, **Subudhi AW**, **Lovering AT**, **Roach RC**, **Vesin JM**, **Kayser B.** AltitudeOmics: baroreflex sensitivity during acclimatization to 5,260 m. *Front Physiol* 9: 767, 2018. doi:10.3389/fphys.2018.00767.
27. **Parati G**, **Di Rienzo M**, **Bertinieri G**, **Pomidossi G**, **Casadei R**, **Groppelli A**, **Pedotti A**, **Zanchetti A**, **Mancia G.** Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood

pressure monitoring in humans. *Hypertension* 12: 214–222, 1988. doi:10.1161/01.hyp.12.2.214.
28. **Kamath MV**, **Fallen EL.** Correction of the heart rate variability signal for ectopics and missing beats. In: *Heart Rate Variability*, edited by Malik M, Camm AJ**.** Futura Publishing Co. Inc, 1995, p. 75–85.
29. **Choi A**, **Shin H.** Quantitative analysis of the effect of an ectopic beat on the heart rate variability in the resting condition. *Front Physiol* 9: 922, 2018. doi:10.3389/fphys.2018.00922.
30. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 93: 1043–1065, 1996.
31. **Faul F**, **Erdfelder E**, **Lang A-G**, **Buchner A.** G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 39: 175–191, 2007. doi:10.3758/bf03193146.
32. **Hissen SL**, **Sayed KE**, **Macefield VG**, **Brown R**, **Taylor CE.** The stability and repeatability of spontaneous sympathetic baroreflex sensitivity in healthy young individuals. *Front Neurosci* 12: 403, 2018. doi:10.3389/fnins.2018.00403.
33. **Manferdelli G**, **Narang BJ**, **Bourdillon N**, **Debevec T**, **Millet GP.** Baroreflex sensitivity is blunted in hypoxia independently of changes in inspired carbon dioxide pressure in prematurely born male adults. *Physiol Rep* 12: e15857, 2024. doi:10.14814/phy2.15857.
34. **Hainsworth R**, **Drinkhill MJ**, **Rivera-Chira M.** The autonomic nervous system at high altitude. *Clin Auton Res* 17: 13–19, 2007. doi:10.1007/s10286-006-0395-7.
35. **Somboon P**, **Kulapatana S**, **B. Watanapa W**, **Chattaris T**, **Sirivongrangson P**, **Livikorn P**, **Nonthadid T**, **Hussarin P.** Heart rate variability and baroreflex sensitivity: factors and reference ranges. *Siriraj Med J* 75: 713–724, 2023. doi:10.33192/smj.v75i10.264037.
36. **Burtscher M**, **Gatterer H**, **Faulhaber M**, **Burtscher J.** Acetazolamide pre-treatment before ascending to high altitudes: when to start? *Int J Clin Exp Med* 7: 4378–4383, 2014.
37. **Leaf DE**, **Goldfarb DS.** Mechanisms of action of acetazolamide in the prophylaxis and treatment of acute mountain sickness. *J Appl Physiol (1985)* 102: 1313–1322, 2007. doi:10.1152/japplphysiol.01572.2005.
38. **Wang J**, **Ke T**, **Zhang X**, **Chen Y**, **Liu M**, **Chen J**, **Luo W.** Effects of acetazolamide on cognitive performance during high-altitude exposure. *Neurotoxicol Teratol* 35: 28–33, 2013. doi:10.1016/j.ntt.2012.12.003.
39. **Garske LA**, **Brown MG**, **Morrison SC.** Acetazolamide reduces exercise capacity and increases leg fatigue under hypoxic conditions. *J Appl Physiol (1985)* 94: 991–996, 2003. doi:10.1152/japplphysiol.00746.2001.
40. **McClure TS**, **Phillips J**, **Koutnik AP**, **Coleman K**, **Chappe E**, **Cutter GR**, **Egan B**, **Norell T**, **Stubbs BJ**, **Bamman MM**, **Kernagis D.** Ketone monoester attenuates declines in cognitive performance and oxygen saturation during acute severe hypoxic exposure under resting conditions. *Exp Physiol* 109: 1672–1682, 2024. doi:10.1113/EP091794.
41. **Poffé C**, **Ramaekers M**, **Bogaerts S**, **Hespel P.** Bicarbonate unlocks the ergogenic action of ketone monoester intake in endurance exercise. *Med Sci Sports Exerc* 53: 431–441, 2021. doi:10.1249/MSS.0000000000002467.
42. **Stubbs BJ**, **Cox PJ**, **Evans RD**, **Santer P**, **Miller JJ**, **Faull OK**, **Magor-Elliott S**, **Hiyama S**, **Stirling M**, **Clarke K.** On the metabolism of exogenous ketones in humans. *Front Physiol* 8: 848, 2017. doi:10.3389/fphys.2017.00848.
43. **Bohr C**, **Hasselbalch K**, **Krogh A.** Ueber einen in biologischer Beziehung wichtigen Einfluss, den die Kohlensäurespannung des Blutes auf dessen Sauerstoffbindung übt. Skandinavisches Archiv Für Physiologie. *Skandinavisches Archiv Für Physiologie* 16: 402–412, 1904. doi:10.1111/j.1748-1716.1904.tb01382.x.
44. **Ma W**, **Berg J**, **Yellen G.** Ketogenic diet metabolites reduce firing in central neurons by opening K(ATP) channels. *J Neurosci* 27: 3618–3625, 2007. doi:10.1523/JNEUROSCI.0132-07.2007.
45. **Manville RW**, **Papanikolaou M**, **Abbott GW.** M-Channel activation contributes to the anticonvulsant action of the ketone body β-hydroxybutyrate. *J Pharmacol Exp Ther* 372: 148–156, 2020 [Erratum in *J Pharmacol Exp Ther* 372: 264, 2020]. doi:10.1124/jpet.119.263350.
46. **García-Rodríguez D**, **Giménez-Cassina A.** Ketone bodies in the brain beyond fuel metabolism: from excitability to gene expression and cell signaling. *Front Mol Neurosci* 14: 732120, 2021. doi:10.3389/fnmol.2021.732120.
47. **Beidleman BA**, **Tighiouart H**, **Schmid CH**, **Fulco CS**, **Muza SR.** Predictive models of acute mountain sickness after rapid ascent to various altitudes. *Med Sci Sports Exerc* 45: 792–800, 2013. doi:10.1249/MSS.0b013e31827989ec.
48. **Citherlet T**, **Raberin A**, **Manferdelli G**, **Pialoux V**, **Millet GP.** Menstrual cycle does not impact the hypoxic ventilatory response and acute mountain sickness prediction. *Sci Rep* 14: 26087, 2024. doi:10.1038/s41598-024-76404-y.
49. **Dearlove DJ**, **Holdsworth D**, **Kirk T**, **Hodson L**, **Charidemou E**, **Kvalheim E**, **Stubbs B**, **Beevers A**, **Griffin JL**, **Evans R**, **Robertson J**, **Clarke K**, **Cox PJ.** β-hydroxybutyrate oxidation in exercise is impaired by low-carbohydrate and high-fat availability. *Front Med (Lausanne)* 8: 721673, 2021. doi:10.3389/fmed.2021.721673.
50. **Tagliapietra G**, **Citherlet T**, **Raberin A**, **Bourdillon N**, **Krumm B**, **Narang BJ**, **Giardini G**, **Pialoux V**, **Debevec T**, **Millet GP.** Effect of menstrual cycle phase on physiological responses in healthy women at rest and during submaximal exercise at high altitude. *Sci Rep* 14: 27793, 2024. doi:10.1038/s41598-024-79702-7.
51. **Quanjer PH**, **Stanojevic S**, **Cole TJ**, **Baur X**, **Hall GL**, **Culver BH**, **Enright PL**, **Hankinson JL**, **Ip MSM**, **Zheng J**, **Stocks J**; **ERS Global Lung Function Initiative.** Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 40: 1324–1343, 2012. doi:10.1183/09031936.00080312.