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Effects of periodic breathing on sleep at high altitude: a randomized, placebo-controlled, crossover study using inspiratory CO_2

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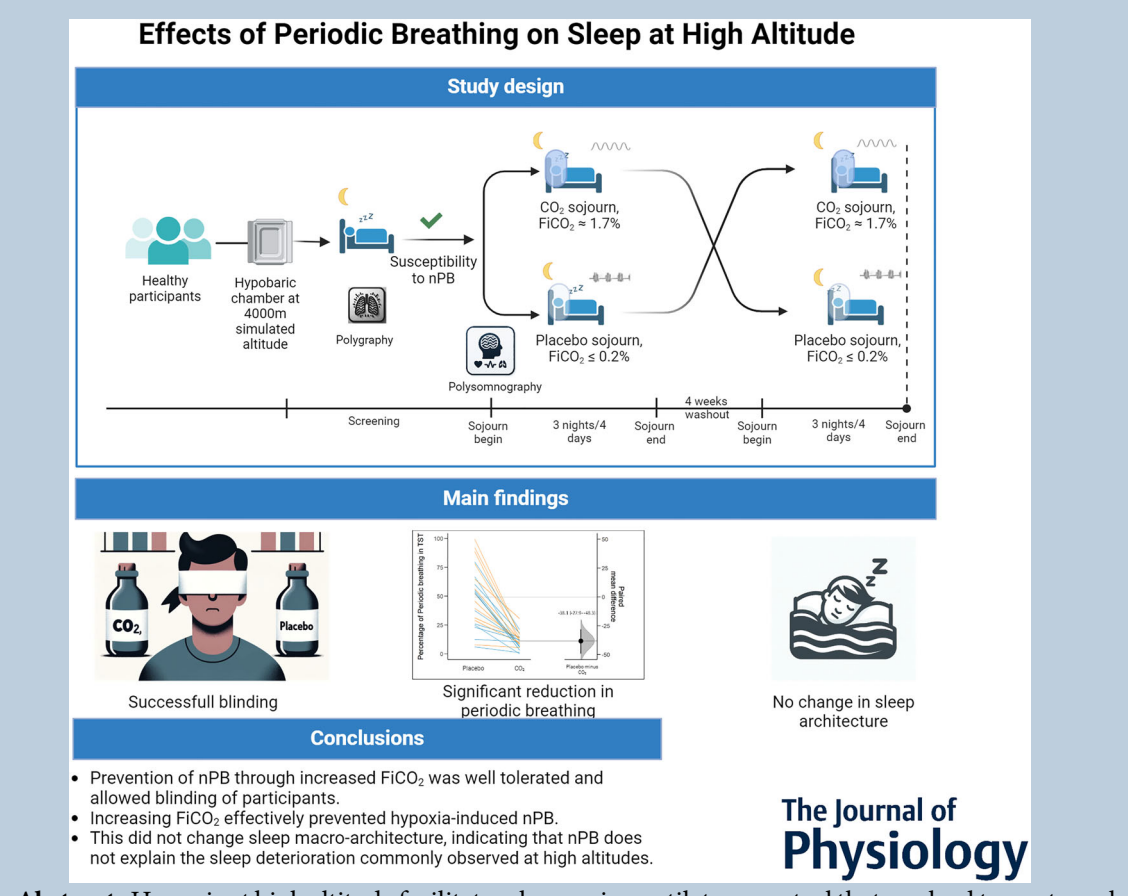

Abstract Hypoxia at high altitude facilitates changes in ventilatory control that can lead to nocturnal periodic breathing (nPB). Here, we introduce a placebo-controlled approach to prevent nPB by

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increasing inspiratory CO_2 and used it to assess whether nPB contributes to the adverse effects of hypoxia on sleep architecture. In a randomized, single-blinded, crossover design, 12 men underwent two sojourns (three days/nights each, separated by 4 weeks) in hypobaric hypoxia corresponding to 4000 m altitude, with polysomnography during the first and third night of each sojourn. During all nights, subjects' heads were encompassed by a canopy retaining exhaled CO_2, and CO_2 concentration in the canopy (i.e. inspiratory CO_2 concentration) was controlled by adjustment of fresh air inflow. Throughout the placebo sojourn inspiratory CO_2 was $\leq$0.2%, whereas throughout the other sojourn it was increased to 1.76% (IQR, 1.07%–2.44%). During the placebo sojourn, total sleep time (TST) with nPB was 54.3% (37.4%–80.8%) and 45.0% (24.5%–56.5%) during the first and the third night, respectively ($P = 0.042$). Increased inspiratory CO_2 reduced TST with nPB by an absolute 38.1% (28.1%–48.1%), the apnoea–hypopnoea index by 58.1/h (40.1–76.1/h), and oxygen desaturation index $\geq$3% by 56.0/h (38.9.1–73.2/h) (all $P < 0.001$), whereas it increased the mean arterial oxygen saturation in TST by 2.0% (0.4%–3.5%, $P = 0.035$). Increased inspiratory CO_2 slightly increased the percentage of N3 sleep during the third night ($P = 0.045$), without other effects on sleep architecture. Increasing inspiratory CO_2 effectively prevented hypoxia-induced nPB without affecting sleep macro-architecture, indicating that nPB does not explain the sleep deterioration commonly observed at high altitudes.

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Abstract figure legend We aimed to (i) develop a method to prevent hypoxia-induced nocturnal periodic breathing (nPB) via an increased inspiratory CO_2 fraction (F_{iCO_2}) and (ii) use this method to investigate the effects of nPB on sleep architecture at high altitude. Healthy participants susceptible to hypoxia-induced nPB (confirmed during a screening night) underwent two sojourns in a hypobaric chamber simulating 4000 m altitude, one with increased (CO_2 sojourn) and one with normal (placebo sojourn) nocturnal F_{iCO_2}, and with polysomnography during the first and third night. The increased F_{iCO_2} effectively reduced nPB without significantly changing sleep macro-architecture. Created with BioRender.com.

Key points

- Periodic breathing is common during sleep at high altitude, and it is unclear how this affects sleep architecture.
- We developed a placebo-controlled approach to prevent nocturnal periodic breathing (nPB) with inspiratory CO_2 administration and used it to assess the effects of nPB on sleep in hypobaric hypoxia.
- Nocturnal periodic breathing was effectively mitigated by an increased inspiratory CO_2 fraction in a blinded manner.
- Prevention of nPB did not lead to relevant changes in sleep architecture in hypobaric hypoxia.
- We conclude that nPB does not explain the deterioration in sleep architecture commonly observed at high altitude.

Introduction

A common complaint of sojourners to high altitude (HA) is disturbed sleep and a feeling of breathlessness upon awakening (Bloch et al., 2015; Weil, 1985). The latter is putatively linked to changes in ventilatory control in response to hypoxia (Burgess et al., 2004): during sleep at altitudes >2500 m, a breathing pattern characterized by repeated central apnoeas interspersed with brief periods of hyperventilation often emerges, termed nocturnal periodic breathing (nPB) (Bloch et al., 2015). While at sea level a similar breathing pattern (Cheyne–Stokes breathing) occurs in heart failure, where it is associated with a worse prognosis (Lanfranchi et al., 1999), the consequences of nPB at HA are poorly explored.

To investigate the consequences of nPB at HA, a method that efficiently prevents nPB in a placebo-controlled manner is required. One approach to reduce nPB

at HA is acetazolamide administration (Liu et al., 2017), which increases and stabilizes ventilation by inducing metabolic acidosis. Intravenous acetazolamide combined with dobutamine was also shown to reduce nPB by increasing cerebral blood flow (Burgess et al., 2018). However, acetazolamide effects persist for up to 24 h (Ritschel et al., 1998) and can thus affect measurements conducted during daytime. An alternative approach to prevent nPB is mildly increasing inspiratory CO_2 concentration (F_{iCO_2}) (Berssenbrugge et al., 1983). Although at high concentrations CO_2 can cause rapid breathing, tachycardia and impaired consciousness, at low concentrations it has no such side effects (Langford, 2005). Moreover, an increase in F_{iCO_2} can be temporally restricted to the night, thus allowing investigation of the isolated consequences of nPB on the ensuing day. While F_{iCO_2} can be increased with dead space masks (Patz et al., 2013), such masks are not well tolerated, and make individual titration of F_{iCO_2} and subject blinding difficult. Accordingly, our first aim was to develop a method to prevent nocturnal nPB via increased F_{iCO_2} in a placebo-controlled manner that maximizes participant comfort.

The second aim was to use this method to investigate the effects of nPB on sleep architecture at HA. An increase in Stage 1 sleep (N1) (Joern et al., 1970; Johnson et al., 2010; Natani et al., 1970), reductions in slow wave sleep (N3) (Joern et al., 1970; Johnson et al., 2010; Natani et al., 1970; Nicholson et al., 1988; Panjwani et al., 2007), rapid eye movement (REM) sleep and sleep efficiency (Nicholson et al., 1988; Panjwani et al., 2007), as well as an increase in sleep latency (Panjwani et al., 2007), are common at HA. While it seems intuitive that nPB contributes to these adverse changes, there is limited evidence for this: although nPB may promote awakening (Shogilev et al., 2015) and/or arousal (Khoo et al., 1996), studies have failed to detect a correlation between nPB and changes in sleep architecture at HA (Graf et al., 2022; Johnson et al., 2010; Nussbaumer-Ochsner, Ursprung et al., 2012). Here, we tested the hypothesis that prevention of nPB increases sleep efficiency, N3 and REM sleep percentages and reduces arousals at HA.

Methods

Ethical approval

This study was approved by the Ethics Committee of Bolzano (No. 76-2021) and conducted in accordance with the *Declaration of Helsinki* (except for registration in a database). Thirty-nine healthy, non-smoking lowlanders (nine women) gave written informed consent to study participation. All experiments took place in a hypobaric chamber (terraXcube, Eurac Research, Bolzano, Italy; internal dimensions 12 × 6 × 5 m) at a barometric pressure of 462 mmHg (corresponding to an altitude of ∼4000 m).

Subjects

A specific inclusion criterion was susceptibility to hypoxia-induced nPB, defined as an apnoea–hypopnoea index (AHI) of ≥30 events/h during a screening night in the chamber (see below). An additional inclusion criterion for women was adherence to hormonal contraception, which aimed to minimize variations in ovarian hormones between measurements. Exclusion criteria were any known sleep-related breathing disorders, previous severe episodes of HA illness, pregnancy, chronic medication or exposure to altitudes >2000 m within the 4 weeks preceding and throughout the study. Seventeen subjects (including all women) did not display susceptibility to nPB and were hence excluded. Another nine subjects withdrew consent to participation after the screening night, and one subject withdrew consent after completing only one sojourn, leaving a total of 12 male subjects who completed all the experiments (mean ± SD; 27.7 ± 4.6 years, 1.79 ± 0.07 m, 71.4 ± 6.3 kg, BMI 22.1 ± 1.9 kg/m^2).

Protocol

Figure 1 illustrates the study protocol. Subjects first underwent a general clinical examination, including structured assessment of sleep disorders. The latter included a face-to-face interview with a medical doctor

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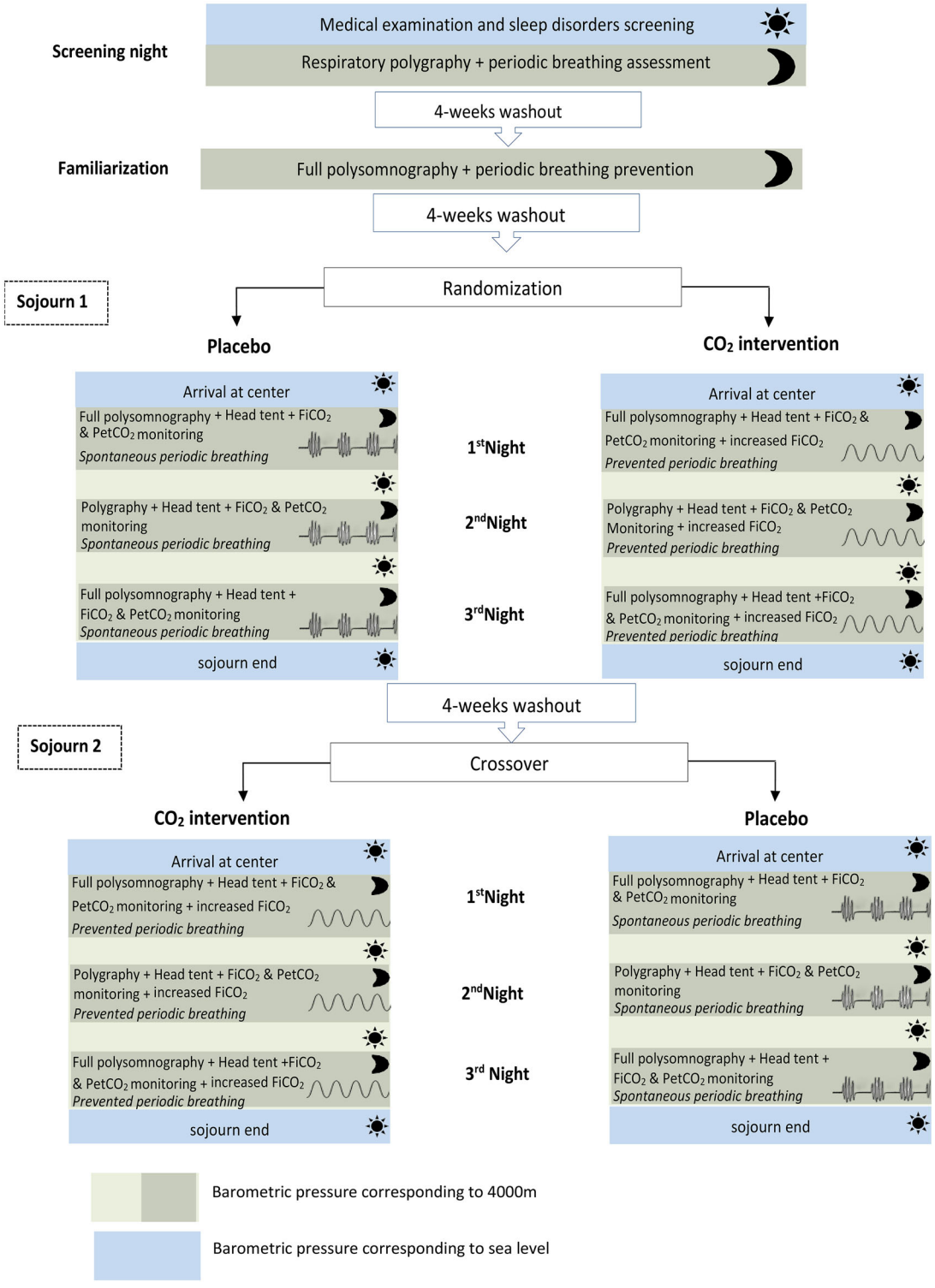

Figure 1. Overview of the study protocol
$FiCO_2$, inspiratory CO_2 fraction; $PetCO_2$, end-tidal CO_2 partial pressure.

who was board-certified in sleep medicine, where all sleep diagnoses according to the International Classification of Sleep Disorders, third edition (ICSD-3), main categories and major subcategories were assessed. Additionally, the presence of any neurological, psychiatric or medical co-morbidities and medication intake was assessed. After this examination, subjects spent the screening night in the chamber. Decompression started after dinner, and time in bed (TIB) was between 23.00 and 07.00 h. Respiration was monitored throughout the night by polygraphy (Alice PDx, Philips Respironics, Amsterdam, Netherlands), which included cardiorespiratory monitoring (nasal airflow, thoracic and abdominal respiratory movements, pulseometry and peripheral oxygen saturation (S_{pO_2})). Note that 12 subjects were recruited after completion of the screening nights as replacement for excluded subjects. For these subjects, susceptibility to nPB was evaluated during the familiarization night (see next paragraph) according to the same criteria as used during the screening nights.

Four weeks after the screening night, subjects spent a familiarization night in the chamber. The purpose was to familiarize them with (i) polysomnography (PSG) and (ii) the set-up for nocturnal CO_2 administration. Decompression was initiated in the afternoon, and TIB was from 23.00 to 07.00 h, with subjects instrumented for PSG and in the set-up described under 'Nocturnal set-up and inspiratory CO_2 administration'.

After another washout of 4 weeks, subjects completed two 3-day sojourns in the chamber (with decompression starting on the first day at ∼09.00 h). The two sojourns were again separated by a 4-week washout. Subjects spent all nights (23.00 to 07.00 h) of the sojourns in the set-up described under 'Nocturnal set-up and inspiratory CO_2 administration', with PSG during the first and third night and respiratory polygraphy during the second night (results of the polygraphy are not reported). During one sojourn, the F_{iCO_2} was increased throughout the nights to prevent nPB (CO_2 sojourn), whereas during the other sojourn the nocturnal F_{iCO_2} was normal (placebo sojourn). The order of the sojourns was randomized, and subjects were blinded toward the nocturnal F_{iCO_2}. Apart from the nocturnal F_{iCO_2}, the two sojourns were identical: subjects were provided three meals per day and had *ad libitum* access to water, caffeine-free tea and snacks. Caffeine intake was limited to one coffee in the morning. Subjects did not perform physical exercise, but were allowed to study, work, or enroll in recreational activities. After the third night, the subjects underwent recompression and were discharged.

Nocturnal set-up and inspiratory CO_2 administration

Subjects spent the familiarization night and all nights of the sojourns in a spacious (2.5 × 4 × 3 m), sound and light insulated, wooden container (smartboxx, Bressanone, Italy). Subjects' heads were placed under an airtight canopy (Snowcap Sleeping Canopy, Higher Peak, MA, USA) that retained expired CO_2. The frame of the canopy was secured to the bed frame with screws. To enhance the airtightness of the canopy, its side and back walls were fastened to the bed frame by Velcro straps. We furthermore covered the subjects with a weighted (7 kg) blanket that closely was attuned to their body shape (thus minimizing air spaces) and that was connected to the front wall of the canopy by Velcro straps (Fig. 2). Pilot experiments revealed that with this setting, F_{iCO_2} in the canopy increased to 3.5–4% and was only mildly affected by subject movements. Regulation of F_{iCO_2} within the canopy was then achieved via adjustment of fresh air inflow: a plastic tube was guided through the wall of

Figure 2. Photographic demonstration of the set-up
The image depicts the set-up inside the sound- and light-insulated wooden container, placed inside the hypobaric chamber. It contains a camera for video polysomnography as well as a bed equipped with a canopy, and weighted blanket. Tubes for fresh air inflow and CO_2 control are seen projecting into the canopy. © Eurac Research – Annelie Bortolotti.

the chamber, which, given the hypobaria in the chamber, provided a continuous inflow of air that was monitored by a digital flowmeter and manually controlled by a needle valve. This tube was connected to a port in the frame of the canopy, from where the air was released through a nozzle into the canopy. A further tube was hung from the top of the canopy (behind the participants face), which sampled gas from within the canopy and fed it to high-precision analysers that continuously measured F_{iCO_2} and the inspiratory O_2 fraction (F_{iO_2}). These sensors were also programmed to trigger an acoustic alarm if F_{iCO_2} in the canopy exceeded 5%. The same alarm could be triggered by the subjects via a 'distress button' placed in the canopy.

During the placebo sojourn, the airflow into the canopy was 60 l/min, which was the highest flow that did not produce distinguishable noise. Nevertheless, pilot experiments revealed that with this airflow F_{iCO_2} in the canopy still increased to ∼0.5%. To further increase CO_2 washout, the transparent side panels of the canopy used for the placebo sojourn were partially removed, whereafter the F_{iCO_2} in the canopy could be maintained at ≤0.2%. This modification was disguised by covering the side panels of the canopies with black, air-permeable netting. During the CO_2 sojourn, the airflow into the canopy was reduced, leading to an increase in F_{iCO_2}. A researcher monitoring both the F_{iCO_2} in the canopy and the subjects' breathing pattern continuously adjusted the airflow to find and maintain the individual lowest F_{iCO_2} that prevented nPB. This researcher could furthermore manually administer bursts of 100% CO_2 into the canopy, which allowed rapid restoration of F_{iCO_2} after leakage induced by excessive subject movement and/or increasing F_{iCO_2} beyond the levels achieved with retention of exhaled CO_2 alone.

PSG and analysis of sleep data

Whole-night PSG was performed with a mobile device (Morpheus recorder light, Micromed, Italy) according to the current guidelines of the American Academy of Sleep Medicine (AASM) (Berry, 2020). Recorded channels included horizontal electrooculography, six-channel electroencephalography, surface electromyography of the mental, submental, splenius capitis muscles, both flexor digitorum superficialis, and anterior tibialis muscles, cardiorespiratory monitoring (electrocardiography, oronasal airflow and thermal sensor, tracheal microphone, S_{pO_2}, thoracic and abdominal respiratory movements) and time-synchronized digital videography. In addition, end-tidal CO_2 partial pressure (P_{ETCO_2}) was continuously measured by a capnograph (CAP10, Medlab GmbH, Stutensee bei Karlsruhe, Germany) and fed as a time-synchronized signal into the PSG software.

Sleep stages were manually scored according to the current AASM criteria (Berry, 2020) by a blinded, board-certified scorer. Here, hypopnoeas are scored if the peak signal excursions drop by ≥30% of pre-event baseline using nasal pressure, and there is either a ≥3% oxygen desaturation from pre-event baseline or the event is associated with an arousal. Apnoeas are scored if there is a drop in peak signal excursion by ≥90% using the oronasal thermal sensor. However, the AASM scoring manual does not specify how to score hypoxia-induced nPB, which has a different morphology from apnoeas/hypopnoeas at sea level or Cheyne–Stokes breathing (Kupper et al., 2008). Accordingly, respiratory events were additionally scored following criteria commonly used at HA (Nussbaumer-Ochsner et al., 2010; Tan et al., 2020). The main differences between the two scoring criteria are: (i) according to the AASM manual, a minimum of 10 s duration is required for a respiratory event, whereas the HA criteria allow scoring respiratory events longer than 5 s, as long as a periodic breathing pattern is evident; (ii) the AASM manual requires the presence of ≥3% oxygen desaturation or associated arousal for scoring hypopnoeas, while the HA criteria do not, provided there is a 50% reduction in the respiratory signal. The drop in respiratory signal by ≥90% was still required to score apnoeas. Periodic leg movements during sleep (PLMS) were calculated according to AASM criteria using validated software (Stefani et al., 2017).

Statistics

Statistical analyses were performed with R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) and IBM SPSS Statistics V.26 for Windows (IBM Corp., Armonk, NY, USA). Only the 12 participants who completed both sojourns were included in the final data analysis. For each sojourn, the sleep and respiratory features were compared as follows: (i) first *vs*. third night within each sojourn and (ii) placebo sojourn *vs*. CO_2 sojourn nights (first placebo *vs*. first CO_2, third placebo *vs*. third CO_2).

Data distributions were analysed with the Shapiro–Wilk test. Variables were predominantly non-normally distributed; therefore, summary data are expressed as median (interquartile range, IQR). Wilcoxon's signed-rank test was used for significance testing of the sleep and respiratory parameters between the nights and between the two scoring methods. For comparison of the two scoring methods for respiratory events (AASM manual and HA criteria), all respiratory parameters, regardless of night or sojourn, were pooled. The effect size was calculated as a Z statistic divided by the square root of the sample size. To estimate the 95% confidence interval around the estimated mean difference between the placebo and CO_2 sojourn (CO_2 administration effect) for the respiratory and sleep

architecture variables, bootstrap resampling (set to 5000 samples) was used. A non-parametric, bias-corrected and accelerated (BCa) bootstrap confidence interval was calculated (Efron & Narasimhan, 2020); this was then visualized with the Gardner–Altman plot. Additionally, a random slope random intercepts generalized mixed linear effect regression model (GLMM) was used to perform adjusted analyses (adjusted for age and BMI) investigating the treatment effect of CO_2 intervention; subjects were considered a random effect, while the night (first or third) was a fixed effect. The relationship between the AHI and S_{pO_2} was investigated with a linear regression model. Two-tailed P-values <0.05 were considered statistically significant. P correction for pairwise comparison of the nPB in the different sleep stages was done with the Benjamini–Hochberg procedure. Additionally, sample size calculations were carried out for the change of sleep architecture in the placebo *vs*. CO_2 sojourn and the effect sizes (f^2) of the unadjusted GLMM models were used for calculations.

Results

Increasing inspiratory CO_2 in a placebo-controlled manner

Nocturnal inspiratory gas concentrations are presented in Table 1. During the placebo sojourn, F_{iCO_2} and F_{iO_2} averages over the duration of the nights were 0.13% (0.07%−0.14%) and 20.7% (20.5%−21.2%), respectively. During the CO_2 sojourn, the F_{iCO_2} required to prevent nPB was 1.76% (1.07%−2.44%), with individual values ranging from 0.17% to 3.1%. This F_{iCO_2} was similar between the first and third night ($P = 0.733$) and corresponded to an inspiratory partial pressure of CO_2 (P_{iCO_2}) of 7.3 (4.4−10.1) mmHg. The F_{iO_2} was 20.1% (19.4%−20.5%).

All participants tolerated the increased F_{iCO_2} well. When asked at the end of the sojourns whether they had been exposed to the CO_2 or to placebo, 46% of the participants guessed wrongly, 29% guessed correctly and 25% could not tell, thus confirming successful blinding.

Nocturnal ventilation

Respiratory parameters measured during the first and third night of the sojourns are presented in Table 1 (scored according to the AASM manual) and Table 2 (scored according to the HA criteria). During the placebo sojourn, the percentage of TST with nPB scored with the HA criteria was 54.3% (37.4%−80.8%) on the first night, and 45.0% (24.5%−56.5%) on the third night ($P = 0.042$). During the CO_2 sojourn, it was decreased to 12.9% (9.3%–14.6%) on the first night and 8.3% (5.2%–2.7%) on the third night (first *vs*. third night: $P = 0.084$, placebo *vs*. CO_2 sojourn $P < 0.001$). Additionally, AHI in TST and TIB, time and percentage of oxygen desaturation index (ODI) ≥3% in TST, ODI ≥4% in TST, and total duration of respiratory events in TST were markedly reduced during the CO_2 *vs*. the placebo sojourn in both the first and the third night ($P < 0.001$ for all variables). Individual differences (95% confidence interval) in selected respiratory parameters (scored with the HA scoring criteria) between the sojourns are illustrated in Fig. 3*A* (Gardner–Altman plot) and Table 3 (values derived from non-adjusted and adjusted GLMM are presented).

During the placebo sojourn, but not during the CO_2 sojourn AHI in TST and TIB, ODI ≥4% and ODI ≥3% were higher and total duration of respiratory events longer during the first *vs*. the third night, (see Table 1 for P-values and effect sizes). There was a negative correlation between the AHI and S_{pO_2} pooled from both sojourns ($R = -0.40$, $P = 0.005$, Fig. 4). A representative tracing of the respiratory channels from the PSG during the placebo and CO_2 sojourn is shown in Fig. 5.

A comparison between the respiratory parameters scored with the AASM criteria and the HA criteria is shown in Table 4. Respiratory events (except obstructive AHI) scored with the HA criteria were higher ($P < 0.001$) compared to the AASM criteria but shorter in duration.

Sleep architecture

Table 5 illustrates sleep architecture variables during both sojourns. There were no differences between the placebo and the CO_2 sojourn, except on the third night, where N3 sleep (%TST) was slightly higher in the CO_2 than in the placebo sojourn ($P = 0.045$), while N2 sleep (%TST) was lower ($P = 0.026$). Individual differences in sleep architecture between the sojourns are shown in Fig. 3*B* (Gardner–Altman plot). The sleep efficiency averaged over all nights was 84.7% (76.6%−90.0%), with no difference between the placebo and the CO_2 sojourn (first night $P = 0.973$, third night $P = 0.556$). Sample size calculations assessing the difference between the placebo and CO_2 sojourn indicated a power of 0.70, 0.77, 0.74 and 0.67, for N1, N2, N3 and REM sleep, respectively. For a power of 0.80 in all sleep stages, at least 16 participants would have been required.

Regarding differences between the nights within a sojourn, N1 and N2 sleep (%TST) were higher during the first compared to the third night, whereas N3 and REM sleep (%TST) were lower in the first *vs*. third night of both sojourns (see Table 5 for P-values and effect sizes).

Figure 6 illustrates the distribution of nPB between the different sleep stages. Considering the distribution of nPB in TIB (Fig. 6*A*), most nPB occurred during N2 sleep. However, when comparing the occurrence of nPB as a

Table 1. Respiratory parameters scored according to the AASM scoring manual

Variable	Placebo sojourn				CO_2 sojourn				Placebo *vs.* CO_2			
	First night (n = 12)	Third night (n = 12)	First *vs.* third night *P*	ES	First night (n = 12)	Third night (n = 12)	First *vs.* third night *P*	ES	First *vs.* first night *P*	ES	Third *vs.* third night *P*	ES
AHI in TST (ev/h)	68.3 (53.2–116.9)	49.4 (31.1–64.2)	**0.021**	0.66	14.4 (9.9–20.3)	15.0 (7.2–16.1)	0.677	0.14	**<0.001**	0.88	**<0.001**	0.88
AHI in REM sleep (ev/h)	46.2 (32.0–62.8)	36.3 (31.7–42.7)	0.424	0.25	24.7 (18.5–32.9)	24.1 (15.9–33.5)	0.733	0.11	**0.016**	0.68	**0.003**	0.79
AHI in NREM sleep (ev/h)	70.2 (52.5–123.7)	50.5 (29.3–72.4)	**0.026**	0.63	13.7 (8.8–18.4)	10.5 (6.2–14.4)	0.610	0.16	**<0.001**	0.88	**<0.001**	0.88
Central AHI in TST (ev/h)	67.2 (38.2–89.9)	38.4 (12.3–62.6)	**0.036**	0.62	8.7 (1.1–18.3)	8.8 (0.5–15.5)	1.000	0.01	**<0.001**	0.88	**0.001**	0.84
Obstructive AHI in TST (ev/h)	0 (0–0)	0 (0–0)	0.181	0.50	0 (0–0)	0 (0–0)	0.174	0.50	—	—	0.789	—
Apnea index in TST (ev/h)	14.2 (3.4–52.0)	8.1 (0.9–15.2)	0.129	0.45	0.3 (0–1.1)	0.1 (0–0.6)	0.358	0.26	**0.002**	0.88	**0.006**	0.79
Hypopnoea index in TST (ev/h)	42.3 (19.9–53.8)	28.1 (24.4–43.5)	0.077	0.52	12.5 (9–17.8)	13.5 (7.1–15.9)	0.724	0.11	**0.001**	0.84	**<0.001**	0.88
Total duration of respiratory events (min)	92.7 (73.3–153.7)	72.2 (52.2–84.5)	**0.034**	0.61	22.8 (18.7–34.4)	24.6 (17.3–34.7)	0.424	0.25	**<0.001**	0.88	**<0.001**	0.88
Mean duration of apnoeas (s)	11.8 (11.1–12.4)	11.5 (11–12.1)	0.142	0.44	11.6 (11.2–12.9)	11 (10.9–11)	0.875	0.18	0.779	0.12	0.057	0.91
Mean duration of hypopnoeas (s)	13.7 (12.7–14.8)	14.8 (13.3–15.2)	**0.049**	0.58	15.7 (14.2–17)	17.9 (14.9–18.3)	0.239	0.35	**<0.001**	0.86	**0.002**	0.88
Hypopnoeas associated with desaturation (ev/h)	41.6 (19.6–53.4)	27.7 (24.2–40.8)	0.077	0.52	12.4 (8.4–16)	11.3 (6.9–15.3)	0.677	0.14	**0.001**	0.84	**<0.001**	0.88
Hypopnoeas associated with arousals (ev/h)	0.2 (0–0.85)	0.3 (0.1–0.7)	0.766	0.10	0.3 (0–0.6)	0.5 (0.2–1)	0.722	0.14	0.813	0.09	0.910	0.05
ODI ≥4% in TST (ev/h)	52.0 (37.7–86.8)	30.45 (16.3–59.2)	**0.026**	0.63	7.0 (3.4–9.2)	5.2 (2.9–6.5)	0.410	0.25	**<0.001**	0.88	**<0.001**	0.88
ODI ≥3% in TST (ev/h)	77.7 (59.0–108.2)	49.1 (34.4–78.5)	**0.016**	0.68	14.9 (9.5–19.1)	12.4 (8.1–16.8)	0.424	0.25	**<0.001**	0.88	**<0.001**	0.88
Mean S_{pO_2} in TST (%)	76.0 (74.5–79.5)	79.4 (75.5–81.4)	**0.030**	0.64	77.8 (76–80.9)	81.2 (80.3–82.8)	**0.003**	0.79	0.061	0.52	0.120	0.44
Mean S_{pO_2} in NREM sleep (%)	76.1 (73.3–79.4)	79.4 (74.5–81.5)	**0.042**	0.59	77.6 (75.8–80.6)	81.2 (80.1–82.8)	**0.003**	0.79	0.108	0.48	0.204	0.38

(*Continued*)

Table 1. (Continued)

Variable	Placebo sojourn				CO_2 sojourn				Placebo *vs.* CO_2			
	First night (n = 12)	Third night (n = 12)	First *vs.* third night *P*	ES	First night (n = 12)	Third night (n = 12)	First *vs.* third night *P*	ES	First *vs.* first night *P*	ES	Third *vs.* third night *P*	ES
Mean S_{pO_2} in REM sleep (%)	73.9 (70.4–76.1)	76.0 (69.0–79.8)	0.099	0.51	75.5 (73.3–78.5)	80.0 (74.8–81.2)	**0.012**	0.76	0.114	0.52	0.071	0.53
Percentage of time S_{pO_2} <90% in TST (%)	100 (99.8–100)	100 (99.7–100)	0.779	0.15	100 (100–100)	100 (100–100)	0.269	0.31	0.341	0.37	0.752	0.20
Percentage of time S_{pO_2} <80% in TST (%)	94.1 (54.4–98.1)	53.7 (16.2–95.4)	**0.008**	0.78	83.3 (33.1–98.2)	10.9 (0.7–40.4)	**0.009**	0.72	0.233	0.36	0.110	0.48
Percentage of time S_{pO_2} <70% in TST (%)	0.1 (0–6.2)	0 (0–1.6)	0.447	0.30	0 (0–0.1)	0 (0–0)	0.100	0.57	0.161	0.43	0.584	0.27
Mean heart rate in TST (bpm)	61.2 (56.5–71.6)	61.7 (56.5–66.5)	0.308	0.31	64.2 (55.2–72.1)	62.8 (55.8–69.3)	0.622	0.16	0.970	0.02	0.666	0.14
Average P_{ETCO_2} in TST (mmHg)	29.9 (28.2–31.7)	28.5 (27.6–29.2)	**0.001**	0.84	31.4 (30.3–34.5)	29.9 (28.9–32.1)	**<0.001**	0.88	**0.009**	0.77	**0.001**	0.84
Average F_{iCO_2} in TST (%)	0.1 (0.1–0.1)	0.1 (0.0–0.1)	**0.037**	0.61	1.9 (1.2–2.2)	1.6 (1.2–2.4)	0.791	0.09	**<0.001**	0.88	**<0.001**	0.88
Average F_{iO_2} in TST (%)	20.7 (20.5–21.2)	20.7 (20.5–21.0)	0.358	0.31	19.6 (19.4–20.5)	20.2 (19.3–20.5)	0.683	0.09	**0.006**	0.89	**0.005**	0.86
Respiratory rate in TST (/min)	21.2 (20.3–21.8)	20.8 (19.8–22.0)	0.530	0.193	20.9 (20.0–23.0)	20.6 (19.9–21.2)	0.129	0.453	0.733	0.113	0.450	0.238

Values are expressed as median (IQR); Wilcoxon signed-rank test is used for significance testing; *P*-values < 0.05 are marked in bold. Abbreviations: AASM, American Academy of Sleep Medicine; AHI, apnoea-hypopnoea index; bpm, beats per minute; ES, effect size; ev/h, events per hour; ODI, oxygen desaturation index; REM, rapid eye movement; S_{pO_2}, peripheral oxygen saturation; TST, total sleep time.

Table 2. Respiratory parameters scored with HA criteria

	Placebo sojourn				CO_2 sojourn				Placebo *vs.* CO_2			
Variable	First night (*n* = 12)	Third night (*n* = 12)	First *vs.* third night *P*	ES	First night (*n* = 12)	third night (*n* = 12)	First *vs.* third night *P*	ES	First *vs.* first night *P*	ES	Third *vs.* third night *P*	ES
AHI in TST (ev/h)	81.9 (54.2–122.7)	56.5 (36.5–84.9)	**0.021**	0.66	17.8 (13.5–23.7)	16.3 (10.2–18.8)	0.266	0.34	**<0.001**	0.88	**<0.001**	0.88
AHI in REM sleep (ev/h)	51.9 (32.6–74.4)	41.3 (35.3–47.8)	0.424	0.25	26.3 (19.7–36)	25.1 (18.2–35.7)	0.791	0.09	**0.016**	0.68	**0.001**	0.84
AHI in NREM sleep (ev/h)	82.2 (53.4–130.9)	56.8 (35.2–95.6)	**0.034**	0.61	17.5 (14.2–22.2)	12.3 (8.1–16.7)	0.151	0.43	**<0.001**	0.88	**<0.001**	0.88
Central AHI in TST (ev/h)	81.9 (54.2–122.4)	56.5 (36.5–84.9)	**0.021**	0.66	17.7 (13.5–23.7)	16.2 (10.2–18.8)	0.301	0.32	**<0.001**	0.88	**<0.001**	0.88
Obstructive AHI in TST (ev/h)	0 (0–0)	0 (0–0)	—	—	0 (0–0)	0 (0–0)	—	—	—	—	—	
Apnoea index in TST (ev/h)	16.7 (6.2–71.3)	13.2 (1.5–30.8)	0.092	0.50	1.2 (0.3–2.9)	0.6 (0.4–1.3)	0.209	0.37	**<0.001**	0.88	**<0.001**	0.79
Hypopnoea index in TST (ev/h)	43.2 (23.3–57)	30.4 (26–48.6)	0.077	0.52	15.2 (10.8–20.5)	14.5 (9.1–18.3)	0.470	0.23	**0.002**	0.82	**<0.001**	0.88
Total duration of respiratory events (min)	107.6 (76.2–162.5)	83.0 (58.4–97.8)	**0.042**	0.59	25.9 (21.3–39.6)	26.5 (18.2–37.5)	0.230	0.37	**<0.001**	0.88	**<0.001**	0.88
Mean duration of apnoeas (s)	11.1 (10.1–12.5)	10.4 (9.9–11)	0.130	0.45	9.6 (8.2–10.9)	9.3 (8.2–10.1)	0.683	0.19	**0.021**	0.66	**0.037**	0.61
Mean duration of hypopnoeas (s)	13.7 (12.5–14.8)	14.5 (13.3–15.2)	0.070	0.53	15.2 (13.9–16.6)	17.4 (14.7–18.1)	0.136	0.44	**0.003**	0.87	**0.002**	0.88
Hypopnoeas associated with desaturation (ev/h)	42.1 (22.2–54.9)	28.5 (24.7–41.7)	0.077	0.52	12.9 (8.5–16.4)	11.5 (7.5–15.5)	0.677	0.14	**0.001**	0.84	**<0.001**	0.88
Hypopnoeas associated with arousals (ev/h)	1.2 (0.6–1.6)	1.2 (0.3–2.1)	0.575	0.14	2.1 (1.3–2.9)	1.3 (0.5–2.5)	0.197	0.37	**0.032**	0.61	0.784	0.09
TST Percentage with nPB (%)	54.3 (37.4–80.8)	45.0 (24.5–56.5)	**0.042**	0.58	12.9 (9.3–14.6)	8.3 (5.2–12.7)	0.084	**0.61**	**<0.001**	**0.88**	**<0.001**	**0.88**
Time of nPB in TST (min)	204.9 (134.5–307.0)	167.3 (104.2–213.0)	0.064	0.54	44.3 (36.6–65.4)	30.4 (19.8–54.6)	**0.042**	**0.59**	**<0.001**	**0.88**	**<0.001**	**0.88**

Values are expressed as median (IQR); Wilcoxon signed-rank test is used for significance testing; *P*-values < 0.05 are marked in bold. Abbreviations: AHI, apnoea-hypopnoea index; ES, effect size; ev/h, events per hour; nPB, nocturnal periodic breathing; ODI, oxygen desaturation index; REM, rapid eye movement; TST, total sleep time.

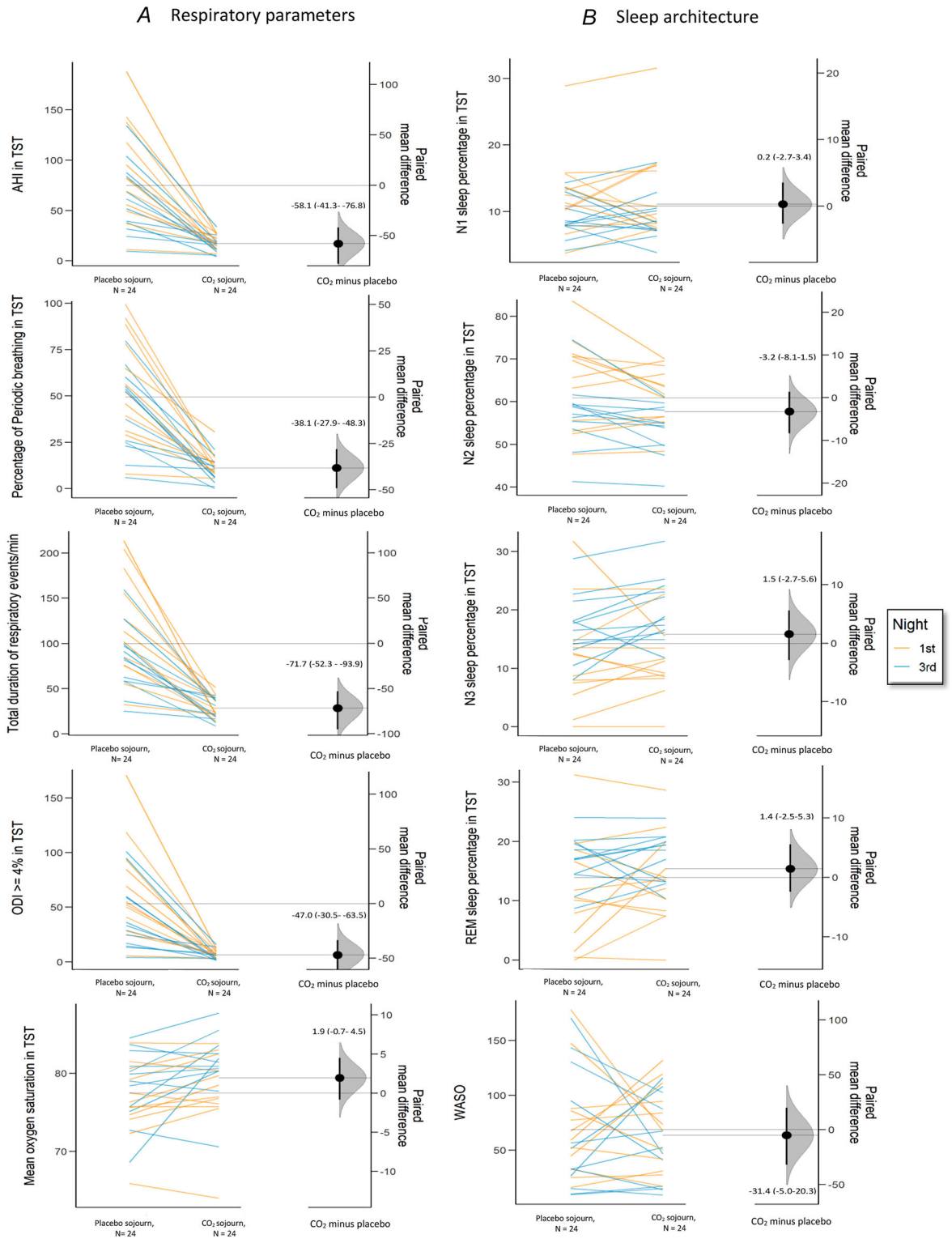

Figure 3. Pairwise comparison of respiratory and sleep architecture variables during the placebo and CO_2 sojourns
Gardner–Altman plot showing estimation the effect of increased inspiratory CO_2 fraction on respiratory (left side) and sleep architecture (right side) variables. The lines colour/shading represent first (orange) and third (blue) night. The variance bar for the effect size is the 95% confidence interval for the difference in the means. Abbreviations: AHI, apnoea–hypopnoea index; ODI, oxygen desaturation index; TST, total sleep time; REM, rapid eye movement; WASO, wake after sleep onset in minutes.

Table 3. Effect of inspiratory CO_2 administration on the respiratory parameters

Variable	Unadjusted mixed effects model (effect 95% CI)	Adjusted mixed effects model (effect 95% CI)
AHI in TST (ev/h)	−58.1 (−75.5 to 40.7)	−58.1 (−76.1 to 40.1)
AHI in REM sleep (ev/h)	−26.0 (−40.6 to 11.4)	−26.0 (−39.8 to 12.2)
AHI in NREM sleep (ev/h	−62.4 (−80.8 to 43.9)	−62.4 (−81.5 to 43.2)
Central AHI in TST (ev/h)	−58.2 (−76.3 to 40.1)	−58.2 (−75.4 to 41)
Obstructive AHI in TST (ev/h)	0 (0–0)	0 (0–0)
Apnoea index in TST (ev/h)	−31.2 (−46.6 to 15.8)	−31.2 (−46.1 to 16.3)
Hypopnoea index in TST (ev/h)	−26.9 (−37.1 to 16.7)	−26.9 (−37.1 to 16.6)
Mean duration of hypopnoeas (s)	2.1 (1.6–2.6)	2.1 (1.6–2.6)
Hypopnoeas associated with desaturation (ev/h)	−27.4 (−37.7 to 17.2)	−27.4 (−37.8 to 17.1)
Hypopnoeas associated with arousals (ev/h)	0.6 (−0.1 to 1.2)	0.5 (−0.1 to 1.2)
ODI $\geq$ 3% in TST (ev/h)	−56 (−73.2 to 38.8)	−56 (−73.2 to 38.9)
Mean oxygen saturation in TST (%)	2.0 (0.4–3.5)	2.0 (0.4–3.5)
Mean oxygen saturation in NREM sleep (%)	2.1 (0.3–3.8)	2.1 (0.3–3.8)
Mean heart rate in TST (BPM)	−0.2 (−3.6 to 3.2)	−0.2 (−3.6 to 3.2)
TST Percentage with nPB (%)	−38.1 (−48.6 to 27.6)	−38.1 (−48.1 to 28.1)
Time of nPB in TST (min)	−144.7 (−182.6 to 106.7)	−144.7 (−181.9 to 107.5)

Treatment effect of CO_2 (95% confidence interval) derived from a generalized mixed linear effect regression model (GLMM); unadjusted, and adjusted for age and BMI. Abbreviations: AHI, apnoea–hypopnoea index; ES, effect size; ev/h, events per hour; nPB, nocturnal periodic breathing; ODI, oxygen desaturation index; REM, rapid eye movement; TST, total sleep time.

Table 4. Differences in the respiratory scoring between the AASM and HA criteria

Variable	AASM scoring, median (IQR) (n = 48)	Alternative scoring, (IQR) (n = 48)	P	ES
AHI in TST (ev/h)	23.4 (12.4–58.2)	26.5 (16.3–68.8)	**<0.001**	0.85
AHI in REM sleep (ev/h)	32.4 (22.4–42.2)	35.05 (23.3–45.2)	**<0.001**	0.75
AHI in NREM sleep (ev/h)	21.9 (10.8–59)	24.85 (13.9–72.4)	**<0.001**	0.85
Central AHI in TST (ev/h)	15.8 (3.2–58.2)	26.5 (16.3–68.8)	**<0.001**	0.87
Obstructive AHI in TST (ev/h)	0 (0–0)	0 (0–0)	0.181	0.25
Apnoea index in TST (ev/h)	0.9 (0.1–10.2)	2.6 (0.6–14.9)	**<0.001**	0.83
Hypopnoea index in TST (ev/h)	20.2 (11.0–33.8)	22 (11.5–34.9)	**<0.001**	0.85
Total duration of respiratory events (min)	39.3 (23.3–79.6)	41.1 (24.8–86.5)	**<0.001**	0.83
Mean duration of apnoeas (s)	11.5 (11–12.2)	10.1 (8.6–11.1)	**<0.001**	0.78
Mean duration of hypopnoeas (s)	14.9 (13.8–16.9)	14.7 (13.5–16.9)	**<0.001**	0.74
Hypopnoeas associated with desaturation (ev/h)	19.0 (10.3–33.6)	19.8 (10.7–33.9)	**<0.001**	0.76
Hypopnoeas associated with arousals (ev/h)	0.3 (0–0.7)	1.3 (0.5–2.5)	**<0.001**	0.85

Values are expressed as median (IQR); Wilcoxon signed-rank test is used for significance testing; P-values < 0.05 are marked in bold. Abbreviations: AHI, apnoea-hypopnoea index; AASM, American Academy of Sleep Medicine; ES, effect size; ev/h, events per hour; HA, high altitude; ODI, oxygen desaturation index; REM, rapid eye movement; TST, total sleep time.

percentage of time spent in the different sleep stages (Fig. 6*B*), nPB occurred mostly in N1 sleep. During the placebo sojourn nights, the central apnoea index was 4.0 (1.3−12.8)/h in REM and 19.1 (4.2−72.4)/h in NREM sleep, respectively (P <0.001).

Figure 7 shows the distribution of nPB throughout the time of the night as a cumulative probability in both sojourns. The incidence of nPB was constant throughout the night.

Discussion

Our approach to prevent hypoxia-induced nPB through an increased F_{iCO_2} was well tolerated, effective and allowed blinding of participants. The high sleep efficiency during both sojourns furthermore indicates that the approach did not interfere with sleep comfort. However, contrary to our hypotheses, the prevention of nPB did not induce relevant changes in sleep architecture.

Table 5. Sleep architecture during the placebo and CO_2 sojourns

	Placebo sojourn				CO_2 sojourn				Placebo *vs.* CO_2			
Variable	First night (n = 12)	Third night (n = 120)	First *vs.* third night *P*	ES	First night (n = 12)	Third night (n = 12)	First *vs.* third night *P*	ES	First *vs.* First night *P*	ES	Third *vs.* third night *P*	ES
TST (min)	403.5 (366.5–424.8)	401.5 (366.8–438.8)	0.906	0.05	407.5 (377.5–426.3)	415.5 (375.8–439)	0.301	0.32	0.937	0.03	0.569	0.18
Sleep efficiency (%)	84.0 (76.3–88.4)	83.5 (76.2–91.4)	0.875	0.06	84.8 (78.5–88.6)	87.0 (78.1–91.3)	0.266	0.34	0.937	0.03	0.556	0.18
Sleep latency (min)	8.3 (5.8–13.9)	11.5 (9.9–22.5)	0.209	0.37	9.3 (2.8–12.4)	9.0 (2.9–20.6)	0.307	0.31	0.556	0.18	0.126	0.45
REM latency (min)	143.5 (108.5–180.5)	84.0 (64.6–105)	**0.032**	0.64	124.5 (69.5–194.8)	96.0 (83.9–106.5)	0.450	0.24	0.492	0.24	0.622	0.16
N1 sleep (%TST)	11.9 (9.6–14.2)	8.2 (7.9–11.1)	**0.042**	0.59	10.2 (8.4–17)	7.9 (7.1–10.2)	**0.009**	0.72	0.519	0.20	0.791	0.09
N2 sleep (%TST)	67.7 (55.0–70.8)	58.0 (55.0–59.4)	**0.012**	0.70	62.6 (56.5–67)	54.6 (49.8–58.2)	**0.001**	0.84	0.301	0.32	**0.026**	0.63
N3 sleep (%TST)	10.6 (7.0–13.9)	17.1 (14.0–19.0)	**0.016**	0.68	10.3 (8.5–14)	18.7 (16.5–23.4)	**0.003**	0.85	0.610	0.17	**0.045**	0.62
REM sleep (%TST)	10.4 (3.8–17.1)	17.1 (14.5–19.7)	**0.025**	0.66	12.9 (8.1–16.6)	19.0 (13.2–20.2)	**0.045**	0.59	0.530	0.19	0.505	0.20
Arousal index (ev/h)	21.3 (13.4–33.0)	15.3 (12.3–19.9)	0.339	0.29	14.8 (12.1–26.2)	15.7 (12.1–19.8)	0.622	0.16	0.233	0.36	0.388	0.26
WASO (min)	63.3 (41.5–86.5)	42.3 (23.6–103.9)	0.147	0.43	70.5 (39.3–98.8)	49.5 (16.5–91.3)	0.054	0.57	0.969	0.02	0.791	0.09
Wake within SPT (%)	13.5 (8.8–19.8)	7.8 (5.6–22.2)	0.077	0.52	14.7 (8.7–21.1)	9.1 (3.6–19.6)	0.077	0.52	1.000	0.00	0.569	0.18
PLMS index TST (ev/h)	5.7 (2.6–11.5)	4.2 (2.2–9.8)	0.875	0.06	3.0 (0–6.4)	2.2 (1.2–10.8)	0.444	0.27	0.789	0.27	0.367	0.27
PLMW index (ev/h)	0 (0–0)	0 (0–0)	1.000	0.289	0 (0–0)	0 (0–0)	1.000	0.289	1.000	0.289	1.000	0.289
PLMS index REM (ev/h)	0 (0–0.8)	1 (0–4.5)	0.571	0.198	0 (0–3.5)	0 (0–1.0)	0.933	0.036	0.496	0.166	0.291	0.372
PLMS index NREM (ev/h)	3.4 (1.8–5.8)	4.4 (1.4–8.6)	0.307	0.329	1.9 (0.0–5.4)	2.4 (0.7–11.7)	0.221	0.375	0.906	0.000	0.910	0.045

Values are expressed as median (IQR); Wilcoxon signed-rank test is used for significance testing; *P*-values < 0.05 are marked in bold. Abbreviations: ev/h, events per hour; NREM, non-rapid eye movement sleep; PLMS, periodic leg movements during sleep; REM, rapid eye movement sleep; SPT, sleep period time; TIB, time in bed; TST, total sleep time; WASO, wake after sleep onset.

Prevention of nPB by inspiratory CO_2 administration

At a simulated altitude of 4000 m, without intervention, the percentage of TST occupied by nPB was ∼50%, which aligns with earlier results collected at 4270 m (Waggener et al., 1984). The principal mechanism underlying hypoxia-induced nPB is an increased chemosensitivity, which induces ventilatory control instability through an elevated *loop gain* (Khoo et al., 1996). This elevated *loop gain* causes ventilatory undershooting or even apnoea in response to transient hypocapnia, and ventilatory overshooting in response to hypopnoea/apnoea-induced hypoxaemia and hypercapnia, thus creating nPB (Ainslie et al., 2013; Dempsey, 2005). The mild increase in F_{iCO_2} in our study led to a 38.1% reduction in the percentage of nPB in TST and a 58.1/h decrease in the AHI, thus exceeding the treatment effect of acetazolamide (Liu et al., 2017). Moreover, the increased F_{iCO_2} had no side effects and allowed for subject blinding as well as restriction of the effect to the night-time. The increased F_{iCO_2} also led to a modest increase in S_{pO_2} despite the resulting reduction in F_{iO_2} and the higher P_{ETCO_2}, which, based on the alveolar gas equation, would *per se* further decrease alveolar P_{O_2} at a given respiratory exchange ratio (Conkin, 2016). This increase in S_{pO_2} was likely explained by a higher pulmonary ventilation and/or by CO_2-induced pulmonary vasodilatation and ensuing improvement in ventilation–perfusion matching (Chuang et al., 2010). Moreover, the prevention of nPB may have contributed to the increased S_{pO_2} during the CO_2 sojourn. While some studies indicate a positive (Lahiri et al., 1983; Salvaggio et al., 1998) or no effect of nPB on S_{pO_2} (Bird et al., 2021), we observed a negative correlation between the AHI and S_{pO_2}, suggesting a negative effect of nPB. The latter interpretation should, however, be made with caution since the negative correlation could also reflect that the likeliness for respiratory events increased with more pronounced hypoxaemia. While the increased S_{pO_2} may have contributed to the nPB prevention by the increased F_{iCO_2}, it likely did not explain the entire effect. The protective effect of the increased F_{iCO_2} against nPB presumably indicated an increased 'CO_2 reserve', i.e. a larger difference between eupnoeic P_{aCO_2} and the threshold P_{aCO_2} below which apnoeas occur (apnoea threshold) (Ainslie et al., 2013).

Prevention of nPB does not change sleep architecture

Sleep in hypoxia was associated with longer REM latency, higher N1 and N2 sleep percentage, and less N3 and

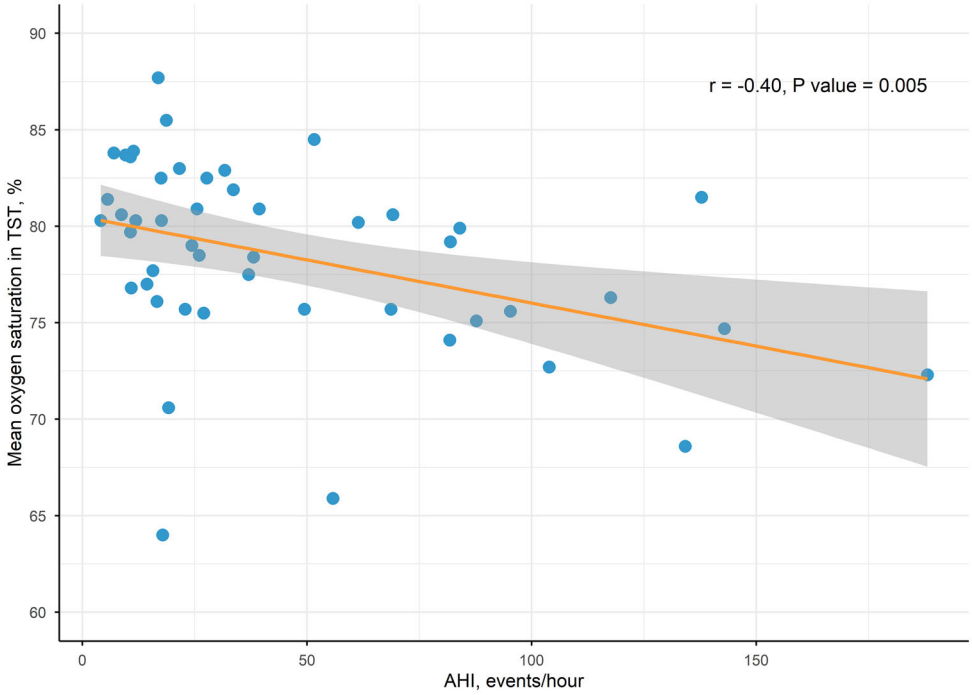

Figure 4. The relationship between the apnoea–hypopnoea index and mean oxygen saturation in total sleep time
The regression line (orange) indicates a negative correlation between AHI (events/hour) and mean oxygen saturation in TST (%), with a higher AHI associated with lower mean oxygen saturation (R = −0.40, P-value = 0.005). Shaded area represents 95% confidence interval for the regression line. The points (blue) displayed on the graph are pooled from 12 participants (48 polysomnographies). Abbreviations: AHI, apnoea-hypopnoea index; R, correlation coefficient; TST, total sleep time.

REM sleep percentage than typically observed in healthy adults in normoxia (Boulos et al., 2019), and the measured values were similar to those reported at altitudes >3500 m (Johnson et al., 2010). Counterintuitively, however, the prevention of nPB barely affected sleep architecture. This is in line with studies at lower altitudes where acetazolamide reduced nPB without improving sleep architecture in healthy subjects (Graf et al., 2022) and in patients with obstructive sleep apnoea syndrome (OSAS) (Nussbaumer-Ochsner, Latshang et al., 2012). Furthermore, during a trek from 1400 to 5000 m, no difference in sleep architecture was detected between subjects who developed nPB and those who did not (Johnson et al., 2010).

Since in animals, F_{iCO_2} supplementation prevented the adverse effects of hypoxia on sleep architecture, it has been suggested that these effects are driven by the hypocapnia associated with the increased pulmonary ventilation in hypoxia (Lovering et al., 2003). However, this is not supported by our results. The absence of a beneficial effect of nPB prevention in our study can also not be explained by more severe hypoxaemia since nocturnal S_{pO_2} was slightly higher during the CO_2 than during the placebo sojourn. Of note, nPB prevention led to an increase in N3 sleep during the third but not the first night. During the first night, this beneficial effect could have been overruled by the 'first night effect', which refers to the deterioration in sleep architecture typically experienced during the initial night in an unfamiliar environment (Byun et al., 2019). This explanation is supported by the observation that in both sojourns sleep architecture was worse on the first compared to the third night. On the other hand, our protocol included a familiarization night to minimize the first-night effect. Whatever the explanation, it should be emphasized that the beneficial effect of nPB prevention during the third night was minimal and probably not of practical relevance.

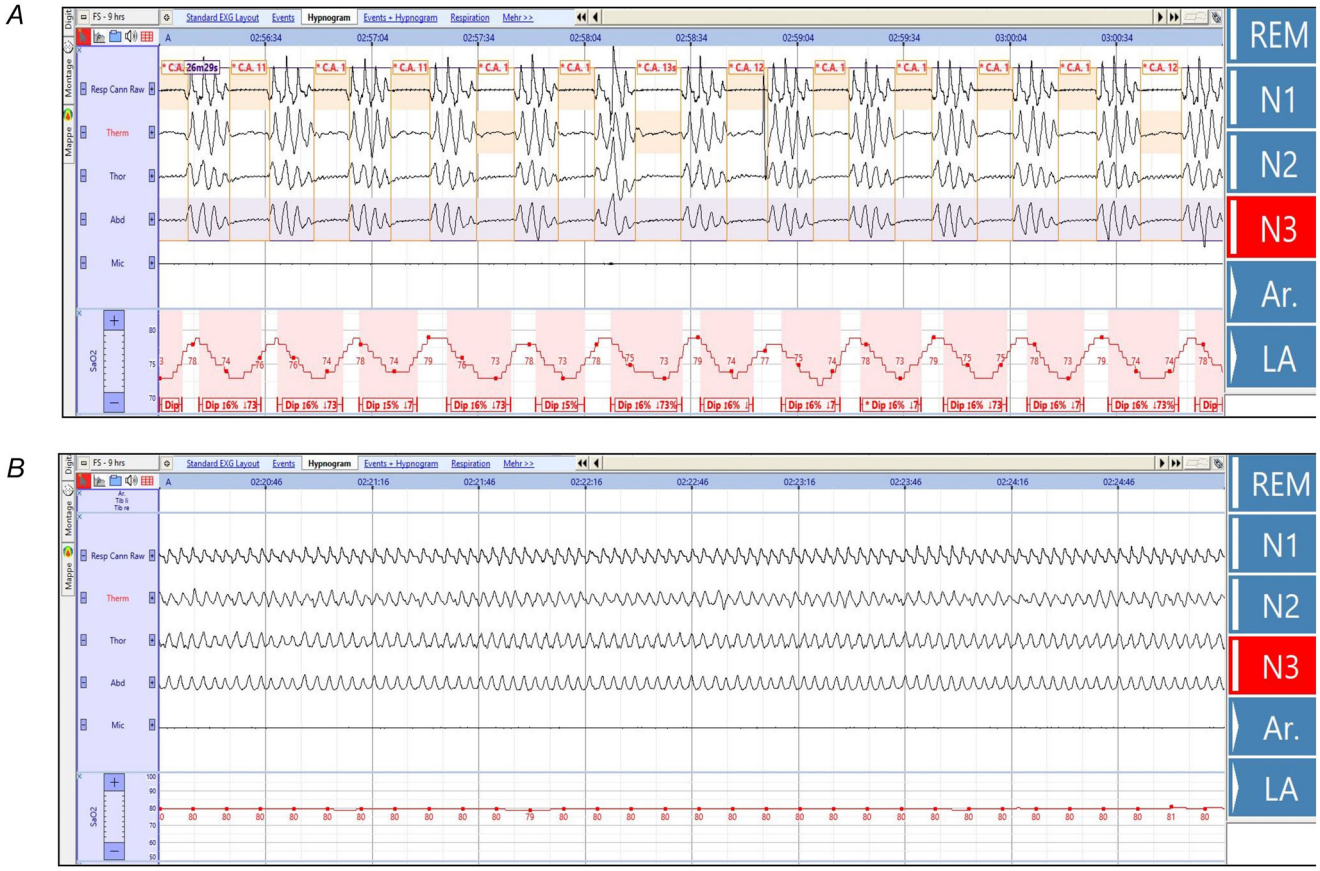

Figure 5. Representative tracing of the respiratory channels from polysomnography during the placebo and CO_2 sojourn
The figure shows a representative 5-min tracing from the same subject in N3 sleep during the placebo (*A*) and the CO_2 sojourn (*B*). Periodic breathing and associated oxygen desaturations are evident throughout tracing *A*, with an average F_{iCO_2} of ∼0.13%. In tracing *B*, the F_{iCO_2} was ∼1.78, which eliminated the periodic breathing. Abbreviations: Abd, abdominal belt; Mic, microphone; Resp Cann, nasal and oral airflow; S_{aO_2}, peripheral oxygen saturation; Therm, thermistor; Thor, thorax belt.

nPB and arousals

A bidirectional relationship between nPB and arousals has been suggested; on one hand, arousals lead to a temporary decrease in P_{aCO_2} that can initiate nPB (Dempsey, 2005). On the other hand, prolonged apnoeas and the strenuous muscular activity during the ensuing hyperventilation may contribute to arousals and thus to disruption of sleep (Khoo et al., 1996). That the latter occurs at HA is, however, not supported by studies reporting marked nPB in the absence of a concomitant increase in arousal index at HA (Graf et al., 2022; Latshang et al., 2013; Nussbaumer-Ochsner, Ursprung et al., 2012). Our results extend these findings by demonstrating that isolated prevention of nPB does not reduce the arousal index. Future research should investigate whether other and more subtle EEG alterations (beyond the traditional AASM-defined arousals) are associated with nPB.

nPB throughout the different sleep stages

In line with a previous HA study (Mizuno et al., 1993), we observed that nPB is not uniformly distributed across sleep stages, but lower in REM and N3 sleep than in N1 and N2 sleep. This is likely due to the sleep-stage dependency of both the CO_2-apnoea threshold (Berssenbrugge et al., 1983; Xi et al., 1993) and the neural motor input to respiratory muscles (Henke et al., 1991). Specifically, it has been suggested that during phasic REM sleep, sporadic rises in central inspiratory drive override the inhibitory effects of hypocapnia on ventilation (Javaheri & Dempsey, 2013) and that the CO_2 reserve is widened in REM compared to NREM sleep (Xi et al., 1993). Notably, while respiratory events occurred during REM sleep, central apnoeas were considerably less common during REM than during NREM sleep, whereas hypopnoeas were more dominant. This presumably reflects that the 'protective' effect of REM sleep on breathing stability can only partially offset the destabilizing effect of the hypoxia associated with 4000 m, resulting in the emergence of hypopnoeas but not apnoeas. In line with this, it was shown that nPB during REM intensifies at 4000 m compared to 3000 m altitude (Mizuno et al., 1993). An alternative explanation is that the respiratory events during REM sleep were triggered by arousals, rather than by hypocapnia. Even though only ∼3% of the respiratory events during REM sleep occurred within 10 s after a detected arousal, we cannot rule out a contribution of more subtle arousals that were not captured by the current scoring method. The lower nPB during N3 sleep presumably reflected a higher autonomic and respiratory stability (McSharry et al., 2013).

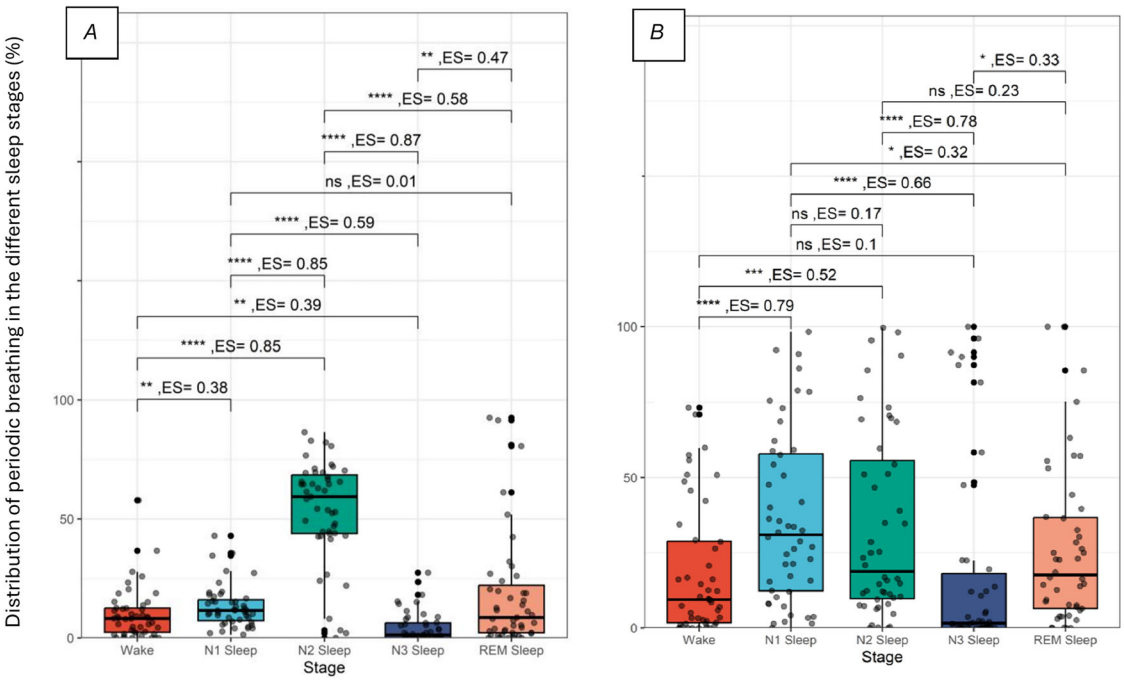

Figure 6. Distribution of periodic breathing in the different sleep stages
A, total nPB. *B*, nPB within the individual stages. Box and whiskers plot representing the distribution of periodic breathing between (*A*) and within (*B*) the different sleep stages. The width of the bar indicates the interquartile range. Wilcoxon signed-rank test was used for significance testing. *P* correction was done with the Benjamini–Hochberg procedure. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < <0.001$; ns, not significant. The points displayed on the graph per sleep stage are pooled from 12 participants (48 polysomnographies). Abbreviation: ES, effect size; REM, rapid eye movement.

Our results also indicate that nPB occurs uniformly throughout the night. This is in line with previous findings showing a similar appearance of nPB early, mid, or late-night at 1500, 3000 and 4000 m (Mizuno et al., 1993).

Acclimatization effect on nocturnal ventilation and sleep architecture

Over the course of the placebo sojourn nPB decreased. Previous HA studies reported inconsistent findings in this respect, showing either that nPB persists/increases (Burgess et al., 2013; Nussbaumer-Ochsner, Ursprung et al., 2012; Salvaggio et al., 1998) or decreases with acclimatization (Latshang et al., 2013; Latshang et al., 2019). It seems likely that the effect of acclimatization on nPB depends on the severity of altitude since the studies where nPB decreased were conducted at considerably lower altitudes than those where nPB persisted or increased further.

A further effect of the short-term acclimatization was an increase in REM and N3 sleep, and a decrease in N1 sleep. These findings are in line with studies showing improvement of sleep architecture despite persistence of nPB after 3 days of acclimatization to 4559 m (Nussbaumer-Ochsner, Ursprung et al., 2012), and after 4 days at 4800 m (Goldenberg et al., 1988). Conversely, no change in sleep architecture was observed over the course of the first week spent at ∼3200 m (Zielinski et al., 2000), suggesting that short-term acclimatization improves sleep architecture only at higher altitudes.

Strengths and limitations

This study has several strengths, including the highly controlled environment in the hypobaric chamber, the placebo-controlled crossover design, and the assessment of sleep with a level I, personnel-attended PSG. Additionally, F_{iCO_2} was individually adjusted to the level required to prevent nPB in a given subject, thus avoiding any nocturnal discomfort that could have led to arousals. A further strength is that we used two different scoring criteria for respiratory events during sleep, one used in clinical settings, and one commonly used in HA studies. Overall, more, but shorter, respiratory events were scored with the HA criteria (Nussbaumer-Ochsner et al., 2010;

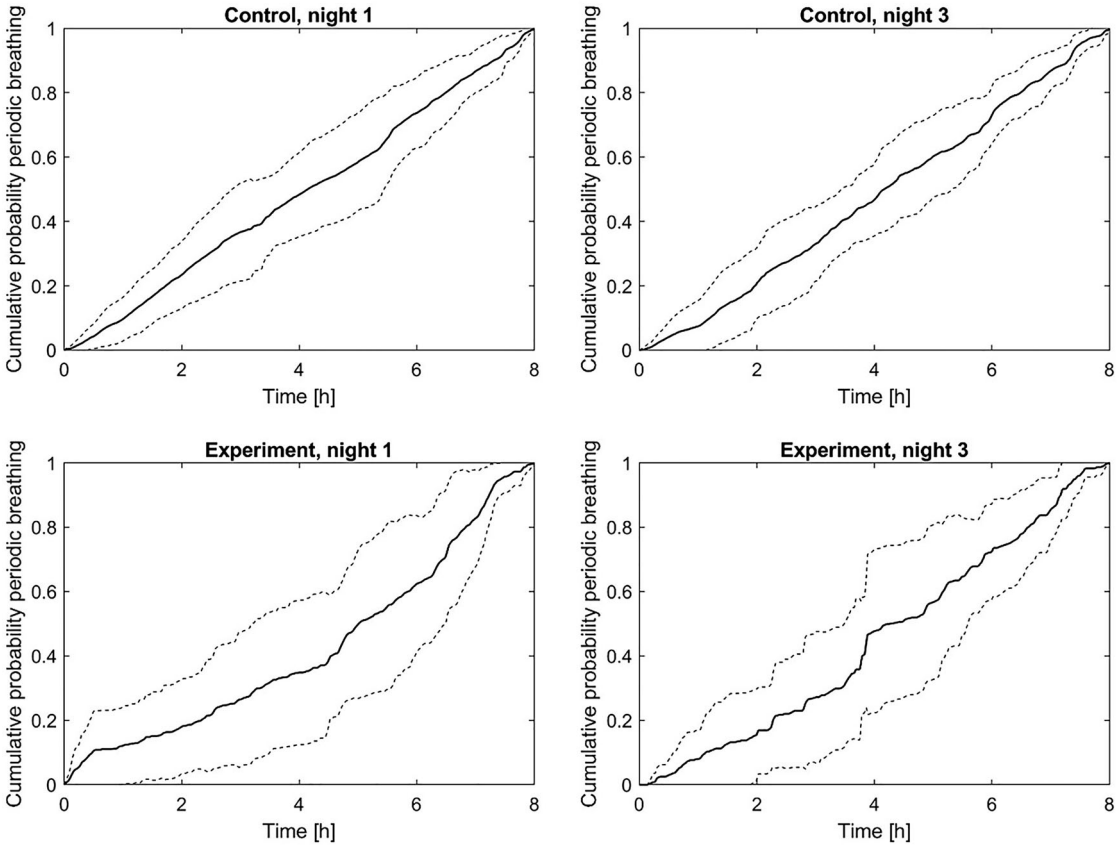

Figure 7. Distribution of periodic breathing throughout the night represented as a cumulative function
Cumulative probability of periodic breathing incidence expressed as a function of night-time. The linear trend represents constant incidence rate. Continuous line represents the mean, and the dashed line the standard deviation.

Tan et al., 2020) than with the AASM manual (Berry, 2020). These differences should be considered when comparing sleep studies at HA with those conducted at sea level.

However, this study also has limitations. First, our sample size was not large. Nevertheless, due to the cross-over, repeated measure design, a total of 48 PSGs could be used for the data analysis. While for respiratory parameters effect sizes were large, four more participants would have been required to reach 0.8 power for the sleep architecture variables. A further limitation is that only men could be included although both men and women were invited to participate. The inclusion criterion of an AHI $\geq$ 30/h at HA during the screening night led to the exclusion of all nine women who consented to participate. This low susceptibility of women to hypoxia-induced nPB is in line with an earlier study where women experienced significant nPB only at 5400 m, but not at 3400 m (Lombardi, 2013).

Conclusions

We demonstrate that F_{iCO_2} can be increased throughout nights spent in hypobaric hypoxia in a placebo-controlled manner, leading to marked stabilization of ventilation during sleep. This effect did not translate into relevant changes in sleep architecture, indicating that nPB is not responsible for the sleep deterioration that typically occurs at HA. Our study provides a new method for blinded nPB prevention that will help elucidate the contribution of nPB to the various effects of HA exposure.

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Additional information

Data availability statement

The data of this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare they have no competing interests.

Author contributions

The experiments were performed in the terraXcube at Eurac Research in Bolzano, Italy. C.S. provided the original idea for the study. A.S., M.C., J.R., H.G., M.F., A.H., B.H. and C.S. had input into the study design and conduct of the study. A.I., A.S. and C.S. wrote the first draft of the manuscript. All listed authors acquired, analysed and interpreted the data. All authors critically revised and approved the final version. All listed authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

acclimatization, carbon dioxide, Cheyne-Stokes, hypobaric, hypoxia, placebo, polysomnography

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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