Supplementary Information

Enhanced Cardiac Vagal Activity and Mood After Low-Dose Hypoxic Gas Inhalation in Healthy Young Adults

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Verification of CVA fluctuations across a range of hypoxic conditions.

In this additional experiment, we monitored changes in cardiac vagal activity (CVA) and percutaneous oxygen saturation (SpO_2) due to acute hypoxia inhalation under two distinct hypoxic conditions. Specifically, in comparison to the primary FIO_2 setting of 13.5% (corresponding to an altitude of 3,500 m) utilized in this study, we also tested milder (FIO_2: 16%, equivalent to 2,000 m) and more severe (FIO_2: 9.6%, equivalent to 6,200 m) hypoxic conditions to identify the optimal conditions for a CVA rebound post-hypoxia.

1) Verification of change in CVA during and after the inhalation of milder hypoxia.

Sixteen healthy young adults, who had no prior exposure to normobaric hypoxia, participated in this study. They were seated at rest on an ergometer and inhaled hypoxic gas (FIO_2: 16%) for 10 minutes (HY^{1-2}). Following the hypoxia exposure, participants rested for 20 minutes in ambient air ($Post^{1-4}$). SpO_2, heart rate (HR), and electrocardiogram (ECG) signals were continuously monitored and recorded throughout each 5-minute segment of the experiment. The root mean square of successive differences (RMSSD) was assessed as an indicator of CVA, with the analysis conducted on natural logarithm (ln) transformed values to adhere to the parametric statistical testing prerequisites. All experiments followed the same protocol as the current study condition, with an FIO_2 of 13.5%. Repeated measures ANOVA was used to evaluate data fluctuations across 7 sessions (Pre, $Hypoxia^{1-2}$, and $Post^{1-4}$). If the sphericity assumption was violated (signaled by Mauchly's test statistic at $p < 0.05$), the Greenhouse-Geisser correction was implemented. Significant ANOVA results led to further analysis with the Bonferroni post-hoc test. Table S1 presents the detailed characteristics of the participants.

Table S1 Anthropological characteristics

Variables	*N* = 16
Gender (male/female)	11 / 5
Age (yr)	24.3 ± 3.1
Weight (kg)	61.1 ± 6.5
Height (cm)	170.5 ± 6.8
BMI (kg/m^2)	21.0 ± 1.5

Values are mean ± standard deviation

BMI Body Mass Index

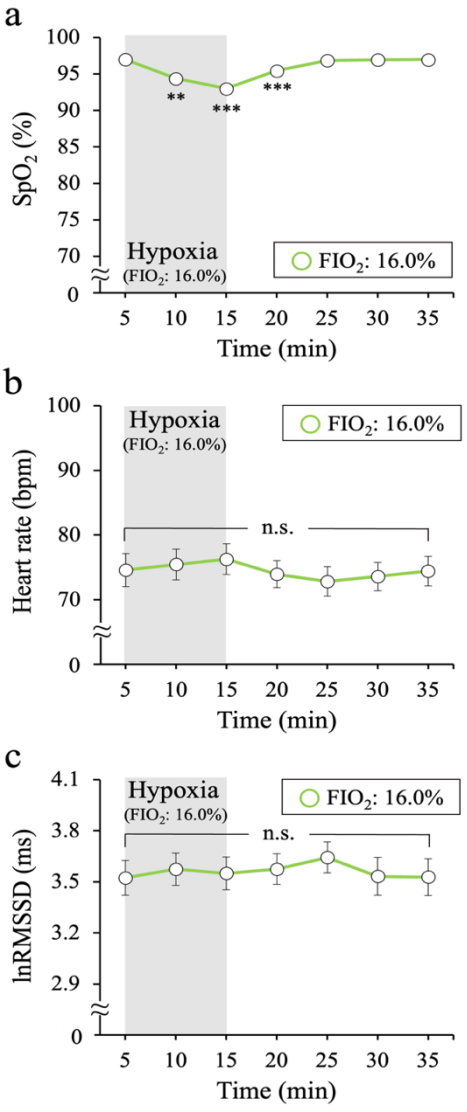

Fig. S1 Fluctuation of SpO_2 (**a**), HR (**b**) and lnRMSSD (**c**) exposure to acute hypoxia (FIO_2: 16%, duration of 10 minutes). $^{**}p <$ 0.01 and $^{***}p < 0.001$ compared to Pre-session. *HY* hypoxia, *HR* heart rate, *n.s.* no significant

A repeated measures ANOVA on SpO_2 indicated a significant main effect (F (1.94, 29.1) = 40.33, $p < 0.001$, $\eta p^2 = 0.729$). Post-hoc comparisons using the Bonferroni correction showed that during hypoxia, the SpO_2 significantly decreased during and after hypoxia compared to baseline (Hypoxia[1]: $p = 0.002$; Hypoxia[2]: $p < 0.001$, Post[1]: $p < 0.001$) (Fig. S1a). No significant differences in session interaction were observed for lnRMSSD and heart rate (Fig. S1b, c).

2) Verification of change in CVA during and after the inhalation of severe hypoxia.

Ten healthy young adults, none of whom had previous experience with normobaric hypoxia, took part in this study. The hypoxic condition was set at an FIO_2 of 9.6%. The experimental procedure was conducted according to a protocol with an FIO_2 of 13.5% for the main study, and the statistical analysis followed a protocol using an FIO_2 of 16% for the first supplementary experiment. Detailed characteristics of the participants are provided in Table S2.

Table S2 Anthropological characteristics

Variables	$N = 10$
Gender (male/female)	4 / 6
Age (yr)	26.7 ± 3.4
Weight (kg)	64.3 ± 13.1
Height (cm)	170.6 ± 8.7
BMI (kg/m^2)	21.9 ± 2.6

Values are mean ± standard deviation

BMI Body Mass Index

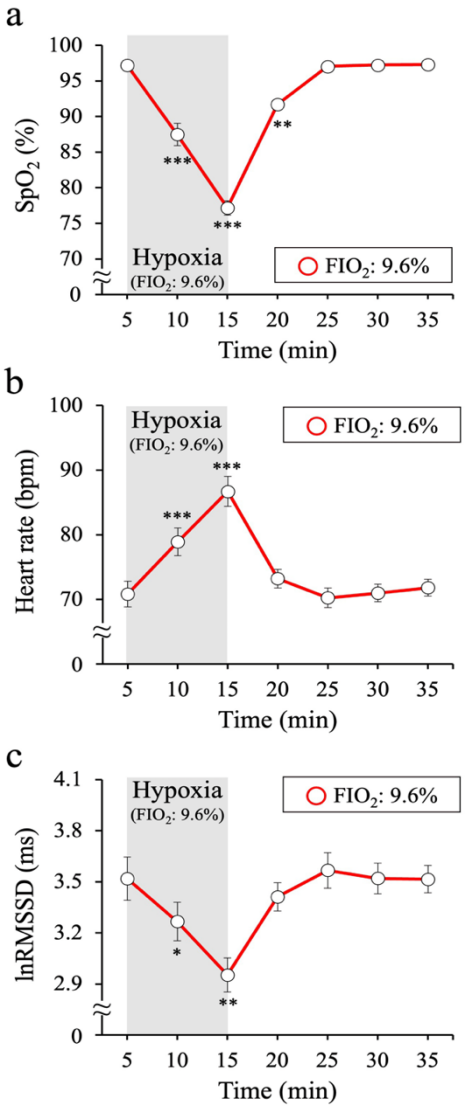

Fig. S2 Fluctuation of SpO_2 (**a**), HR (**b**) and lnRMSSD (**c**) exposure to acute hypoxia (FIO_2: 9.6%, duration of 10 minutes). $^{*}p <$ 0.05, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to Pre-session. *HY* hypoxia, *HR* heart rate

A repeated measures ANOVA on SpO_2 indicated a significant main effect (F (2.00, 18.03) = 112.99, $p < 0.001$, $\eta p^2 = 0.926$). Bonferroni-corrected post-hoc analyses revealed that SpO_2 levels were significantly reduced both during and after hypoxia compared to baseline (Hypoxia^1: $p < 0.001$; Hypoxia^2: $p < 0.001$, Post^1: $p = 0.001$) (Fig. S2a). lnRMSSD showed main effect (F (2.58, 23.25) = 16.55, $p < 0.001$, $\eta_p^2 = 0.648$). Bonferroni-corrected post-hoc comparisons indicated that lnRMSSD significantly decreased during hypoxia compared to baseline (Hypoxia^1: $p = 0.014$; Hypoxia^2: $p = 0.005$) (Fig. S2b). Finally, heart rate exhibited

the main effect (F (6, 54) = 45.55, $p < 0.001$, $\eta_p^2 = 0.835$). Post-hoc comparisons using the Bonferroni correction showed that during hypoxia, the heart rate significantly decreased during hypoxia compared to baseline (Hypoxia[1]: $p < 0.001$; Hypoxia[2]: $p < 0.001$). Following hypoxia, there was no rebound in CVA, or corresponding bradycardia observed (Fig. S2c).