2023-03-16

# The effects of acute hypoxia on cognitive and cardiovascular parameters in healthy subjects

Ravi, R



10.20935/acadbiol6070
Academia Biology
Academia.edu

ACADEMIA BIOLOGY

Research Article
Published: 2023-03-16
https://doi.org/10.20935/AcadBiol6070

# The effects of acute hypoxia on cognitive and cardiovascular parameters in healthy subjects

Rajeevlochan Ravi[1], Mirza M. F. Subhan[1,*]

Academic Editor(s): Georgios Panos and Andre van Wijnen

## Abstract

Background: Hypoxia can affect the health and safety of patients and persons in various occupations. There is uncertainty surrounding the effect acute hypoxia may have on cognition and the cardiovascular system.

Aims: The aim was to examine cognition using the Wechsler Abbreviated Scale of Intelligence – First Edition (WASI-I) and investigate heart rate variability (HRV) with varied fraction of inspired oxygen ($FiO_2$) of 0.12, 0.165, and 0.21.

Methods: Seventeen healthy volunteers participated in two tasks of the WASI-I: block design (BD) and matrix reasoning (MR). BD, MR, and HRV were measured during randomized gas interventions. A subset had their cerebral tissue oxygenation levels (TOI) evaluated.

Results: Cognitive tests for BD ($p$ = 0.133) and MR ($p$ = 0.237) were not significantly different under different $O_2$ concentrations. HRV data showed a decrease in high frequency (HFnu) for MR subset ($p$ = 0.001) with decreasing $FiO_2$. Mean heart rate for BD ($p$ = 0.016) and MR ($p$ = 0.007) increased with decreasing $FiO_2$. NIRS data showed the mean TOI did not significantly change ($p$ = 0.611) during BD; however, during MR, TOI ($p$ = 0.003) decreased with lowering $FiO_2$.

Conclusions: Parasympathetic activity and cerebral tissue oxygenation both fell during MR with increasing hypoxia. The cognitive tests did show decreasing trends, albeit non-significant, with increasing severity of hypoxia. HR also increased during hypoxia for both BD and MR. We suspect cognitive function is related to oxygen saturation ($SpO_2$) levels.

**Keywords:** *hypoxic, cognition, heart rate variability, normal subjects, high altitude*

**Citation**: Ravi R, Subhan MMF. The effects of acute hypoxia on cognitive and cardiovascular parameters in healthy subjects. *Academia Biology* 2023;1. https://doi.org/10.20935/AcadBiol6070

## 1. Introduction

Hypoxia has been known to cause neuropsychological deficits and long-term impairment in concentration, executive function, visual-spatial skills, and memory of affected individuals [1]. Executive functions are cognitive control processes run by the prefrontal cortex, regulating perception, motor responses, planning, and decision making [2], and these functions are affected by hypoxia [3]. Hypoxia exposure can concern very diverse populations in many situations, such as aircrew and mountaineers (altitude exposure), divers, industrial workers [4], and even patients (sleep apnea, chronic obstructive pulmonary disease, carbon-monoxide poisoning, etc.).

Commercial airliners fly at an altitude up to 35,000–40,000 ft (10.7–12 km). However, they maintain a cabin pressure equal to an altitude of 8,000 ft (2.4 km), exposing airline crew and passengers onboard to a fraction of inspired oxygen ($FiO_2$) of 0.148–0.169, for extended periods of time during flying. Pilots are required to practice their spatial abilities, navigation awareness, and visual-motor coordination to respond to danger swiftly so they can map and navigate the airliner to safety [5]. The loss of cabin pressure in an airliner can expose persons to hypobaric hypoxia. This is due to a fall in atmospheric pressure at high altitude decreasing the partial pressure of $O_2$ ($PO_2$). The standard adult range for arterial $O_2$ partial pressure ($PaO_2$) is 12.0–14.6 kPa (90–110 mmHg), and the normal arterial $O_2$ saturation ($SpO_2$) is 94–98% [6]. Cognitive dysfunctions have been reported with a $PaO_2$ below 7.99 kPa (60 mmHg; [1]).

Using the alveolar gas equation, we calculated the estimated alveolar partial pressure of $O_2$ ($P_AO_2$) for three $FiO_2$ gas interventions to be 0.21 = 100 mmHg (13.3 kPa), 0.165 = 68 mmHg (9.0 kPa) and 0.12 = 36 mmHg (4.7 kPa) [7].

Heart rate variability (HRV) corresponds to the sympathetic and parasympathetic nervous systems (SNS and PNS) and can be used to estimate how the autonomic nervous system changes in diverse environments [8]. Hypoxia has been shown to affect the PNS

[1]School of Biomedical Sciences, Faculty of Health, University of Plymouth, Plymouth, United Kingdom
*email: feisal.subhan@plymouth.ac.uk

component at rest in healthy subjects [9], though no studies have investigated the effect of hypoxia on HRV during cognitive testing.

Hypoxia has been shown to cause cognitive impairment [10]. However, another study failed to observe any changes in cognition during hypoxia [11]. Due to these discrepancies above and our intent to identify a test that could effectively assess cognitive skills used by persons exposed to acute hypoxia, we felt the effects of acute hypoxia on cognition required further investigation.

Henceforth, the aim of our study was to assess cognitive function during various acute states of hypoxia and simultaneously investigate autonomic function and cerebral oxygenation in healthy subjects.

# 2. Materials and methods

## 2.1. Ethics approval

The study was approved by the Faculty of Science and Engineering Research Ethics Committee and conformed to the Declaration of Helsinki. The subjects were students of the University of Plymouth and were recruited by convenient sampling. All subjects gave informed and written consent before participation. The experimental protocol was explained to all subjects.

## 2.2. Subjects

In total, 17 healthy subjects (nine male) participated in this cross-sectional study. They were all resident at sea level. The exclusion criteria included subjects who reported any cardiorespiratory disease or color vision deficiency. Subjects' ethnicity was 14 White, two Asian, and one Mixed. Subjects refrained from alcohol, caffeine, and intense exercise for six hours before participation.

## 2.3. Experimental protocol

Height (Seca, Germany) and weight (Marsden, UK) were measured, and then the subjects completed a health questionnaire and had their blood pressure, heart rate, peripheral capillary oxygen saturation ($SpO_2$), spirometry, and HRV measured at rest (**Figure 1**). Laboratory temperature was controlled at 23°C.

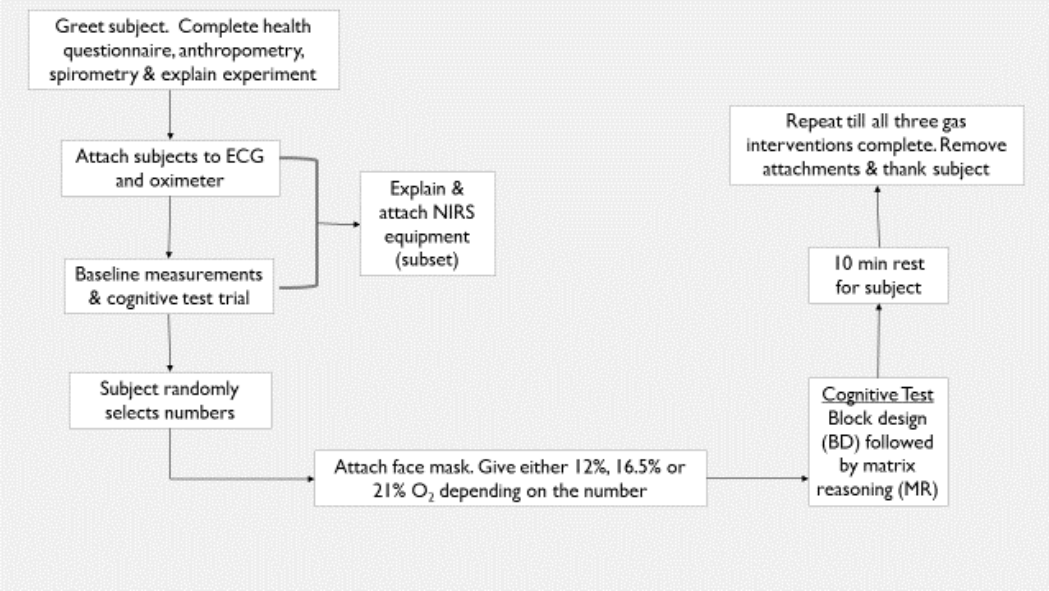



**Figure 1** • The study protocol for cognitive, cardiovascular, and NIRS testing in this study. The subjects were exposed to $FiO_2$ of 0.21, 0.165, and 0.12.

## 2.4. $SpO_2$

All subjects had their $SpO_2$ monitored (Vital Signs monitor 300 series, Welch Allyn, USA) throughout the study for baseline and hypoxic gas effects. If the $SpO_2$ was <80% for more than one minute, the study would be halted. $SpO_2$ was recorded before starting the gas intervention and at the end of cognitive testing.

## 2.5. Spirometry

Before introducing hypoxic gas mixtures, subjects, while seated, performed spirometry (MicroLab, MicroMedical, UK) as a precautionary measure to assess their lung function. The test was repeated to give a minimum of three reproducible blows. The highest spirometric values were calculated as a percentage predicted, relative to reference values. All subjects' spirometric data were within normal limits.

## 2.6. Randomized gas interventions

Subjects were asked to randomly pick three numbered cards (in succession); each number corresponding to a specific $FiO_2$ (0.21, 0.165, and 0.12) and the order picked determined the sequence of gas given during the experiment. This single blind protocol resulted in the subjects not knowing which gas was administered. All three gas interventions were performed on the same day with a 10-minute break between tests (**Figure 1**).

Subjects breathed through a face mask (Hans Rudolf, USA; disinfected on every use), connected through a valve (Hans Rudolf, USA) and tubing to a 150 L Douglas bag (Harvard Apparatus, USA) which was filled with the respective hypoxic gas that needed administering (**Figure 2**). For a $FiO_2$ of 0.21, the valve was connected to an empty Douglas bag out of view and positioned to allow atmospheric air to enter the tubing. Subjects were informed that if they experienced any distress during the experiment, the study would be halted. All 17 subjects completed the study.

**Figure 2 •** The set-up of the BD experiment (taken and used with permissions). A stimulus book and the shapes were placed in front of the subject's line of vision.

### 2.7. Cognition task

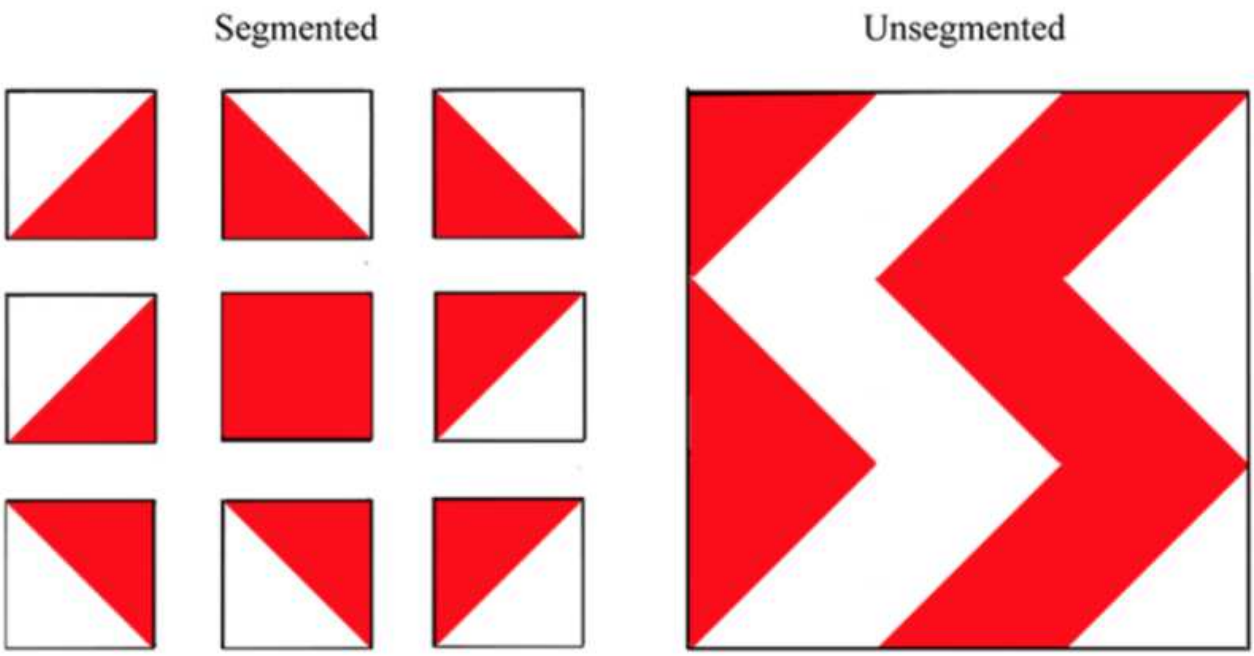
The Wechsler Abbreviated Scale of Intelligence – First Edition (WASI-I) was used for this study and is designed for accurate estimation of an individual's intellectual functioning. It is cost-effective, quick, and reliable [12]. Although there are many methods to assess cognition, we specifically used the WASI-I as we thought it was fit for purpose in motor, spatial, and kinesthetic skills in persons exposed to acute hypoxia (patients, mountaineers, and cabin crew). Virués-Ortega and co-workers have suggested four criteria for high-altitude cognitive tests, covering psychometric properties, ease of administering the test, low practice, and ceiling effects (indicating accuracy) and lastly, shown to be effective at altitude in two studies [3]. We believe the WASI-I fits the first three of these criteria, and our current study wishes to add data for the last criterion.

The four subsets of WASI-I test are vocabulary, block design (BD), matrix reasoning (MR), and similarities. This estimates subjects' general cognitive functioning and provides an approximate intelligence quotient (IQ) score. Vocabulary and similarities tests were not conducted as this required a verbal component and this could not be completed while subjects wore a facemask (**Figure 2**).

The two-subset WASI-I takes approximately 15 minutes. We opted to perform the two-subset which consisted of individually testing the visual performance and general intellectual ability of our subjects, using BD and subsequently MR. These two tests have no verbal component. BD evaluates spatial visualization and reasoning, visual-motor coordination, and abstract conceptualization. In summary, it measures the ability to analyze abstract visual stimuli. Correspondingly, MR measures the non-verbal fluid reasoning, essentially assessing their ability to perceive relationships between abstract symbols and part–whole relationships [12]. Performing two visual-spatial cognitive tests aids deeper understanding, which increases the reliability of conclusions drawn [13].

As per WASI-I instructions, subjects were first provided with a BD and MR trial run from the manual. It required subjects to copy the design for two BD tasks until the design was correct and the MR trial was run without a time limit with two MR tasks. After the gas valve was opened, subjects started the BD test, with the stimulus book and shapes placed in front of them. After completing the shape requested, the time required to complete the BD shape was recorded and then the next stimulus was shown. BD required subjects to rearrange blocks to match a two-dimensional red-and-white geometric pattern shown to them within a specific time frame (between 30 and 120 s). If 120 s elapsed, the subject would be moved on to the next question. Subjects were provided nine cuboid blocks and were required to reproduce 13 designs presented in the WASI-I stimulus book (**Figure 3a**). Each block side was either fully red, fully white, or half red with half white. Subjects were awarded points for successfully re-creating the design. Subjects were awarded points based on attempt number and completion speed. The BD test was discontinued if the subject failed to reproduce the design after two consecutive attempts or exceeded the time limit allocated for the task [13].

*3a*




3b

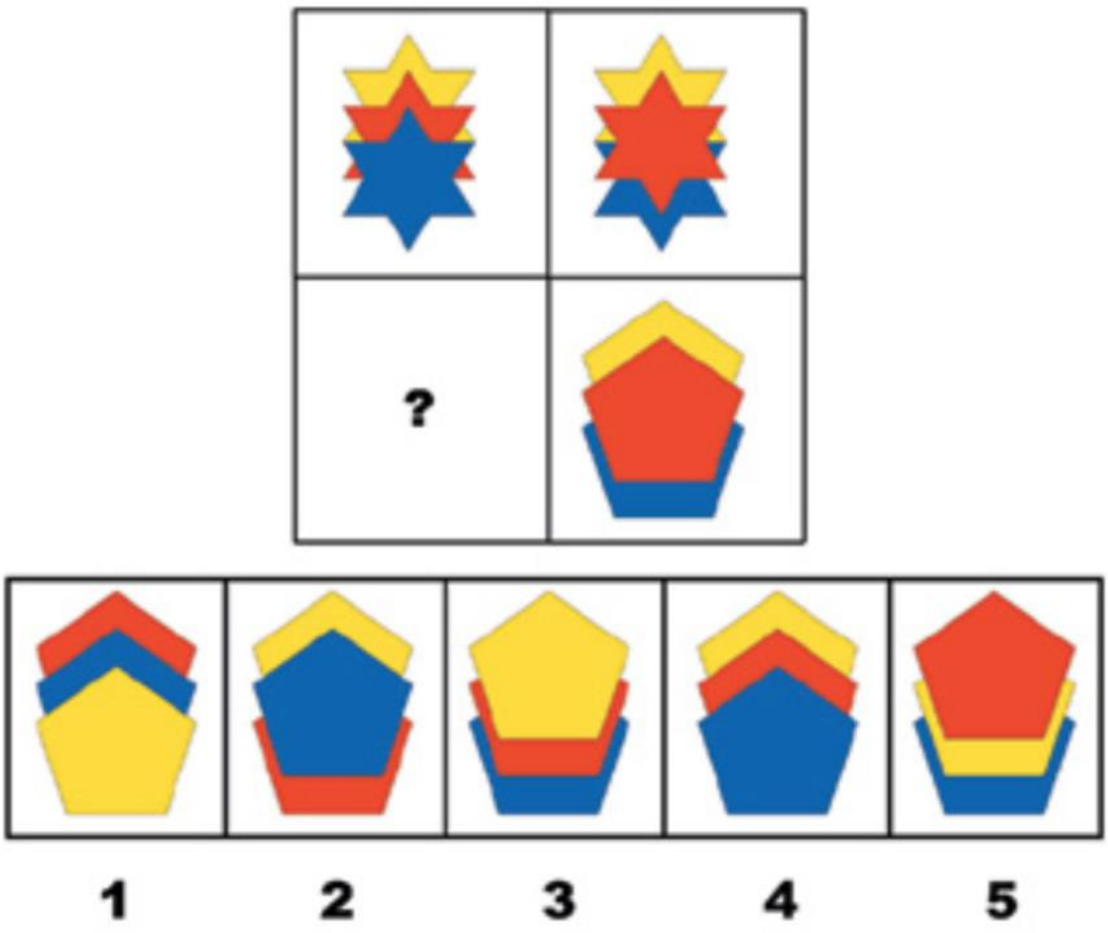



**Figure 3 •** (a) Visual representation of the segmented cuboid blocks with a representative unsegmented design. (b) Example of matrix reasoning section which illustrated five options below and the incomplete matrix to fill, the correct answer in this example is four. (*Wechsler Abbreviated Scale of Intelligence™ (WASI™), Copyright (C) 1999 NCS Pearson, Inc. Used with permission. All rights reserved.*)

The MR test consisted of 35 visually depicted incomplete matrices presented in the WASI-I stimulus book. Subjects viewed an incomplete matrix and pointed to their correct option from five possible options, which they thought correctly matched the incomplete matrix (**Figure 3b**). Each correct answer was recorded by the investigator. If a subject was unable to answer or took more than 30 seconds on any one matrix, they received a score of zero and were made to progress to the next matrix. After four consecutive failures, MR testing was discontinued [13].

Both BD and MR had differential start points based on the test subject's age. All subjects in this study started on the same BD design and MR question. No subject completed the total number of BD or MR tasks, and tests were repeated for each $FiO_2$ gas to determine the difference between the time taken and overall cognitive performance. The raw scores obtained from both tests were converted to T scores, using the WASI-I manual.

### 2.8. Brain oxygenation – near-infrared spectroscopy (NIRS)

A subset of ten subjects (six female) had brain oxygenation levels measured along with all other measurements. Near-infrared spectroscopy (NIRS) (Hamamatsu, Japan) was used for non-invasive real-time assessment of regional (frontal) cerebral oxygen saturation ($rSO_2$), and the ideal location for optode placement was our subjects' foreheads [14]. NIRS is a light-based technology used to detect oxygen in various tissues, and it is increasingly being used to monitor cerebral perfusion [14], including under hypoxic conditions [15]. NIRS uses the principles of optical spectrophotometry where photons are easily capable of penetrating several centimeters of tissue and bone, including the skull [14]. Optode pads were attached to doubled-sided adhesive pads, which were set four centimeters apart. The adhesive pads with a black bandage helped prevent light interference. The tissue oxygenation index (TOI%) was the value of interest from NIRS [14].

### 2.9. Heart rate variability

HRV testing was conducted during cognitive testing under the three gas interventions. HRV data were obtained by placing four pre-gelled disposable electrodes with one on each wrist and one on each ankle. This location is relatively devoid of underlying muscle, thereby reducing interference. The electrodes were connected to a PowerLab 26T, and LabChart 8 HRV analysis software (AD Instruments, Australia) was used to interpret the ECG data. Mean heart rate (HR), standard deviation of normal to normal intervals (SDNN), low-frequency nu (LFnu), and high-frequency nu (HFnu) were some of the HRV parameters used in the analysis.

### 2.10. Statistics

Due to subjects taking different lengths of time to complete the tests, the minimum time taken for all subjects was compared. Therefore, to standardize the analysis of the HRV data, the last three minutes of each BD task was used and the last 1 minute and 30 seconds of each MR was used for each subject. NIRS data were collected every second, and a mean was taken, although the minimum and maximum were also analyzed.

Descriptive analysis for the subjects were calculated using Statistical Package for Social Sciences (SPSS) Version 24 and Microsoft Excel. The cognitive and HRV results were analyzed by repeated measures analysis of variance (ANOVA). The HRV data were normalized using the frequency domain data divided by heart rate squared ($RR^2$) and time domain data by only heart rate (RR; *16*). Wilks' Lambda was used for multivariate testing, and Bonferroni's correction was used to adjust for type 1 errors. A $p$ value < 0.017 was accepted as significant. Pairwise comparisons between the mean were interpreted using the $p$ value. This stricter significance threshold is normally applied when comparing multiple variables over time, as it is likely by chance alone that some of them will be statistically significant. Therefore, if three variables (0.21, 0.165, and 0.12) are compared for any one variable to reach a statistical significance, variables must have a $p$ value equal to 0.05/number [3] of variables = 0.017. Logistic regression was used by considering MR/BD as the dependent variable and with body mass index (BMI), physical activity (PA), LFnu, HFnu, SDNN, and HFnu normalized as independent variables in the model. The significance level was set at 0.05.

## 3. Results

### 3.1. Anthropometric and questionnaire data

The subjects' mean (±SD) age was 21.11 ± 1.22 years and BMI was 22.51 ± 2.58 kg $m^{-2}$. The anthropometric measurements are provided in **Table 1.**

**Table 1 •** The mean, standard deviation, minimum, and maximum for anthropometric and physical activity data of all subjects ($n$ = 17)

| | Mean (±SD) | Minimum | Maximum |
|---|---|---|---|
| **Age (years)** | 21.11 ± 1.22 | 19.00 | 23.00 |
| **Height (m)** | 1.73 ± 0.09 | 1.59 | 1.86 |
| **Weight (kg)** | 67.47 ± 11.84 | 49.00 | 86.00 |
| **BMI (kg $m^{-2}$)** | 22.51 ± 2.58 | 18.74 | 27.40 |
| **Physical activity (number $week^{-1}$)** | 1.76 ± 1.35 | 0.00 | 4.00 |

### 3.2. Time to complete cognitive tests and $SpO_2$

The mean (±SD) time taken for all 51 individual experiments was 579 ± 171 s. This ranged from 298 to 969 s. This time included both the BD and MR components. Mean time to complete each WASI-I test showed no significant change over the three gas interventions ($p$ = 0.15; **Table 2**), although there was a trend for it to increase with decreasing oxygen concentration. Neither time taken for BD ($p$ = 0.27) nor MR ($p$ = 0.18) showed any differences over the three gas interventions. For all three gas interventions, the time taken to complete BD was significantly longer than MR (0.21 $p$ = 0.001; 0.165 $p$ = 0.0001; 0.12 $p$ = 0.01).

As $FiO_2$ decreased, $SpO_2$ values showed a significant difference from baseline values (0.21 $p$ = 0.3; 0.165 $p$ = 0.03; 0.12 $p$ = 0.00003). Baseline values taken before cognitive testing showed no differences between all three tests ($p$ = 0.25). As $FiO_2$ decreased, $SpO_2$ at the end of testing showed an overall significant decrease (**Table 2**) and pairwise comparison also showed significant differences between each gas intervention.

### 3.3. Cognition data

Mean BD T scores showed a dose response through the various $O_2$ interventions (**Table 2**), albeit a non-statistical effect

($p$ = 0.133). Relative to the control, the percentage drop in BD score for 0.165 and 0.12 $FiO_2$ was 1.96% and 4.31%, respectively. Additionally, mean MR T scores illustrated a non-significant effect ($p$ = 0.237). Interestingly, MR T score showed an increase of 1.05% from 0.21 to 0.165, and conversely from 0.21 to 0.12, it declined by 4.72% (**Table 2**).

**Table 2 •** Data showing the mean (±SD) $SpO_2$, time taken for test, BD and MR scores across all three gas interventions administered to the subjects ($n$ = 17) and the overall test within-subjects $p$ value

| Mean data | 21% Oxygen | 16.5% Oxygen | 12% Oxygen | $p$ value across three gas interventions |
|---|---|---|---|---|
| Baseline $SpO_2$ (%) | 97.8 ± 1.01 | 97.4 ± 1.50 | 97.2 ± 1.75 | 0.25 |
| $SpO_2$ end of cognitive test (%) | 98.06 ± 0.56 | 95.82 ± 2.88 | 90.29 ± 5.44 | 0.000007 |
| Total time for testing (s) | 519 ± 139 | 584 ± 197 | 590 ± 175 | 0.15 |
| Time for BD testing (s) | 314 ± 91 | 351 ± 139 | 348 ± 129 | 0.27 |
| Time for MR testing (s) | 205 ± 88 | 233 ± 71 | 242 ± 101 | 0.18 |
| Mean BD raw score | 58.82 ± 8.5 | 56.59 ± 12.1 | 54.65 ± 11.4 | 0.071 |
| Mean BD T score | 59.82 ± 6.04 | 58.65 ± 8.00 | 57.24 ± 7.32 | 0.133 |
| Mean MR raw score | 23.00 ± 3.91 | 23.24 ± 4.89 | 21.94 ± 3.33 | 0.237 |
| Mean MR T score | 44.88 ± 7.95 | 45.35 ± 9.78 | 42.76 ± 6.59 | 0.237 |

## 3.4. HRV data

### *3.4.1. Non-normalized time and frequency domain parameters (BD)*

During BD, HR increased significantly ($p$ = 0.016) as $FiO_2$ decreased (**Table 3**). The percentage change in HR between 0.165 and 0.21 illustrated an increase of 5.32%. In the percentage change between 0.12 and 0.21 $FiO_2$, there was an overall increase of 6.57%. The change in HR from 0.12 to 0.165 $FiO_2$ was only 1.18%. Pairwise comparison data for HR from 0.12 to 0.21 $FiO_2$ showed borderline significance ($p$ = 0.018) during BD. SDNN, SD1, and SD2 showed no significant differences across the three gas interventions. Likewise, for the frequency domain parameters, there were no significant differences in any of the parameters over these three interventions (**Table 3**).

### *3.4.2. Normalized time and frequency domain parameters (BD)*

After performing normalization calculations on the HRV data, no time or frequency domain parameters showed any significant difference across the three gas interventions (**Table 3**).

**Table 3 •** Mean (±SD) non-normalized and normalized time, frequency domain, and non-linear HRV data and TOI for BD over all three gas interventions administered to the subjects ($n$ = 17; nu = normalized units) and the overall test within-subjects $p$ value

| BD mean data | 21% Oxygen | 16.5% Oxygen | 12% Oxygen | $p$ value across three gas interventions |
|---|---|---|---|---|
| TOI (%; $n$ = 10) | 72.21 ± 8.13 | 72.38 ± 8.83 | 71.52 ± 7.81 | 0.611 |
| Non-normalized HRV data | | | | |
| HR (beats $min^{-1}$) | 91.52 ± 11.7 | 96.39 ± 10.60 | 97.53 ± 10.95 | 0.016 |
| SDNN (ms) | 65.97 ± 31.35 | 68.04 ± 36.86 | 68.49 ± 40.23 | 0.865 |
| SD1 (ms) | 41.45 ± 31.71 | 52.16 ± 40.92 | 50.82 ± 42.99 | 0.386 |
| SD2 (ms) | 82.09 ± 35.05 | 79.05 ± 37.70 | 83.77 ± 43.70 | 0.816 |
| Total power ($ms^{-2}$) | 4951.70 ± 5017.93 | 5208.73 ± 6160.57 | 5800.51 ± 8077.21 | 0.795 |
| LF ($ms^{-2}$) | 1657.21 ± 1269.54 | 1246.10 ± 1017.10 | 1311.44 ± 1281.84 | 0.203 |
| LFnu (nu) | 46.52 ± 24.44 | 43.03 ± 21.76 | 51.03 ± 16.93 | 0.397 |
| HF ($ms^{-2}$) | 1878.05 ± 2983.44 | 2248.18 ± 3921.03 | 2223.92 ± 3290.54 | 0.874 |
| HFnu (nu) | 38.98 ± 13.09 | 37.47 ± 15.54 | 37.93 ± 17.78 | 0.912 |
| LF/HF ratio | 1.58 ± 0.92 | 1.57 ± 1.34 | 1.84 ± 1.70 | 0.683 |
| Normalized HRV data | | | | |
| nSDNN | 0.098 ± 0.043 | 0.110 ± 0.065 | 0.109 ± 0.064 | 0.539 |
| nSD1 | 0.061 ± 0.047 | 0.084 ± 0.072 | 0.082 ± 0.071 | 0.245 |
| nSD2 | 0.121 ± 0.046 | 0.126 ± 0.065 | 0.133 ± 0.067 | 0.651 |

| | | | | |
|---|---|---|---|---|
| NLFnu (nu (ms)$^{-2}$) | 0.00012 ± 0.00006 | 0.00011 ± 0.00006 | 0.00013 ± 0.00008 | 0.562 |
| nHFnu (nu (ms)$^{-2}$) | 0.00009 ± 0.00003 | 0.00010 ± 0.00005 | 0.00010 ± 0.00005 | 0.596 |

### *3.4.3. Non-normalized time and frequency domain parameters (MR)*

Throughout MR testing, the mean HR increased after interventions of hypoxic gas, demonstrating a significant effect ($p$ = 0.007; Table 4). The mean HR increased by 3.94% between 0.165 and 0.21 $FiO_2$. From 0.21 to 0.12, it increased by 5.79%. It increased by 1.79% from 0.12 to 0.165. Pairwise comparison data from 0.12 to 0.21 showed a statistical difference ($p$ = 0.010) in HR. No other time domain parameters showed any significant differences across the three interventions. HFnu showed a statistically significant decrease over the three interventions ($p$ = 0.001). Pairwise comparison also showed a significant difference from 0.21 to 0.12, which equated to a 20.57% fall. No other frequency domain parameter showed any significant difference across these three occasions.

**Table 4 •** Mean (± SD) non-normalized and normalized time, frequency domain, and non-linear HRV data for MR and TOI over all three gas interventions administered to the subjects ($n$ = 17; nu = normalized units) and the overall test within-subjects $p$ value

| MR mean data | 21% Oxygen | 16.5% Oxygen | 12% Oxygen | p value across three gas interventions |
|---|---|---|---|---|
| TOI (%; $n$ = 10) | 73.35 ± 7.57 | 72.72 ± 8.57 | 68.96 ± 8.68 | 0.003 |
| Non-normalized HRV data | | | | |
| HR (beats min$^{-1}$) | 88.50 ± 10.91 | 91.99 ± 9.73 | 93.63 ± 11.32 | 0.007 |
| SDNN (ms) | 58.68 ± 31.00 | 56.81 ± 26.60 | 61.70 ± 24.73 | 0.485 |
| SD1 (ms) | 88.50 ± 10.91 | 91.99 ± 9.73 | 93.63 ± 11.32 | 0.310 |
| SD2 (ms) | 73.21 ± 36.17 | 70.90 ± 31.92 | 77.69 ± 34.31 | 0.435 |
| Total power (ms$^{-2}$) | 3919.80 ± 3759.36 | 3259.75 ± 2809.90 | 3073.36 ± 2553.55 | 0.222 |
| LF (ms$^{-2}$) | 1472.43 ± 1575.45 | 1173.00 ± 943.01 | 1336.80 ± 1080.19 | 0.526 |
| LFnu (nu) | 43.72 ± 15.93 | 47.01 ± 17.86 | 49.87 ± 19.65 | 0.353 |
| HF (ms$^{-2}$) | 1566.74 ± 1615.59 | 1373.23 ± 1760.20 | 1593.98 ± 2322.51 | 0.778 |
| HFnu (nu) | 47.83 ± 15.82 | 44.38 ± 16.03 | 38.00 ± 14.91 | 0.001 |
| LF/HF ratio | 1.13 ± 0.80 | 1.40 ± 1.15 | 1.79 ± 1.53 | 0.064 |
| Normalized HRV data | | | | |
| nSDNN | 0.084 ± 0.042 | 0.086 ± 0.040 | 0.095 ± 0.038 | 0.214 |
| nSD1 | 0.054 ± 0.037 | 0.055 ± 0.039 | 0.072 ± 0.062 | 0.238 |
| nSD2 | 0.105 ± 0.049 | 0.107 ± 0.046 | 0.118 ± 0.051 | 0.245 |
| nLFnu (nu (ms)$^{-2}$) | 0.00010 ± 0.00004 | 0.00011 ± 0.00004 | 0.00012 ± 0.00004 | 0.075 |
| nHFnu (nu (ms)$^{-2}$) | 0.00011 ± 0.00005 | 0.00011 ± 0.00004 | 0.00010 ± 0.00005 | 0.263 |

### *3.4.4. Normalized time and frequency domain parameters (MR)*

Normalized time and frequency domain parameters showed no significant differences across the gas three interventions (**Table 4**).

### 3.5. NIRS subset data

Across the three gas interventions, mean TOI did not significantly change for BD ($p$ = 0.611; **Table 3**); in comparison, for MR, it showed a significant decrease ($p$ = 0.003; **Table 4**). However, pairwise comparison data for MR showed no significance. Apart from mean TOI, NIRS also recorded the maximum and minimum TOI values. Across the three gas interventions, BD exhibited no significant change in the maximum ($p$ = 0.558) and minimum ($p$ = 0.404), while MR showed a borderline significant maximum ($p$ = 0.018) and significant minimum ($p$ = 0.003).

### 3.6. Regression data

Logistic regression was carried out on SPSS, and six separate analyses were conducted with the dependent variable as BD 0.12, BD 0.165, BD 0.21, MR 0.12, MR 0.165, and MR 0.21 $FiO_2$. Regression analysis showed no significant result except for MR 0.165, which showed that PA ($p$ = 0.236), HR ($p$ = 0.427), LFnu ($p$ = 0.745), and HFnu ($p$ = 0.886) were not significantly associated. However, SDNN ($p$ = 0.04) was significantly associated with MR 0.165; a linear regression (**Figure 4**) illustrates a borderline significance ($p$ = 0.06). A further linear regression of $SpO_2$ (combined $FiO_2$ with 0.21, 0.165, and 0.12) against BD, MR, and total T score showed no significant relationship ($p$ = 0.46, $p$ = 0.90, and $p$ = 0.54, respectively).

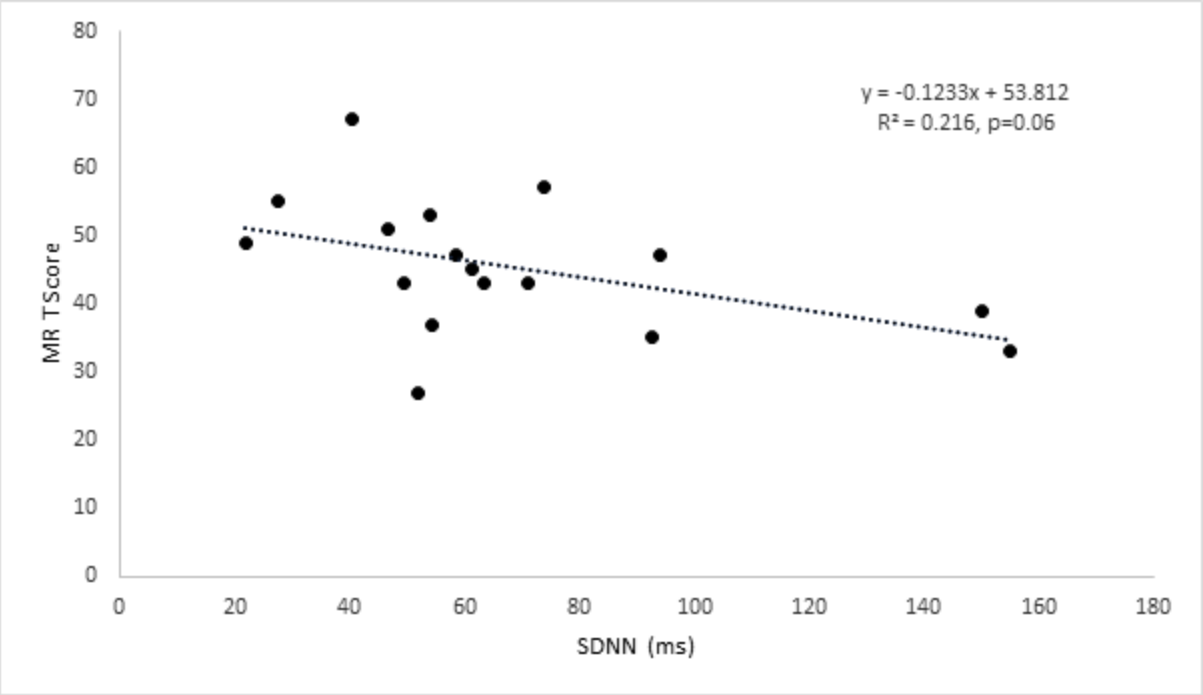



**Figure 4 •** Graph illustrating the linear regression of MR T score at 0.165 $FiO_2$ against SDNN ($n$ = 17).

### 3.7. Adverse effects/symptoms

Five (29%) subjects had a headache after one of the three tests; two had it after the 0.12 $FiO_2$, two had it after 0.21, and one had it after all tests. Two (12%) other subjects felt light-headed during some tests.

## 4. Discussion

Overall, the main findings showed no significant change in the cognitive ability of the participants for either BD or MR between $FiO_2$ interventions of 0.12, 0.165, and 0.21. However, the HR for both BD and MR, HFnu in MR, and TOI levels for MR were statistically significant over the three decreasing gas interventions. Additionally, the regression data illustrated that SDNN was significantly associated with MR for 0.165.

Oxygen concentrations in 22 commercial aircraft flights have been found to range from approximately 15.2% to 17.6% [17]. Therefore, the 0.165 we used in this study was suitable and 0.12 would be more applicable to a higher altitude environment. More recently, it has been noted that a $PaO_2$ of 35–60 mmHg (4.7–8.0 kPa) was an important predictor of cognitive ability [1]. Using the alveolar gas equation [7] for a $FiO_2$ of 0.12, we estimated the $PaO_2$ to be 36 mmHg (4.7 kPa). Although $SpO_2$ values decreased with less oxygen in our study, they often may not fully reflect $PaO_2$ levels, as they can be affected by several factors, including cardiac output.

We chose the WASI-I due to it having necessary non-verbal components related to executive function involving motor kinesthetic skills, which are relevant to persons working at high altitude or with hypoxia, for example, the roles aircraft crew undertake. We thought the WASI-I would be a good representation of visuospatial tasks in these persons, particularly when affected by hypoxia. According to our knowledge, there is no published data using the WASI-I with hypoxia or altitude studies. Furthermore, we could not find any hypoxic studies assessing cognition using a motor kinesthetic component (**Table 5**).

Although our cognitive testing data did not show any difference across the three gas interventions, there was a non-significant dose effect with decreasing $FiO_2$, shown by an increase in total testing time and a decrease in BD score. Additionally, the raw BD score had a borderline $p$ value, if the stricter criteria of $p < 0.017$ was not followed, as done by some researchers [18]. $SpO_2$ levels did show a significant dose effect decrease over the three gas interventions. Therefore, the non-significant trend in BD suggests the possibility that our subjects' ability to analyze abstract visual stimuli decreased with increasing levels of hypoxia. MR did not display a non-significant dose effect as seen with BD; however, the overall value did fall from 0.21 to 0.12 $FiO_2$. MR evaluated our subjects' ability to perceive relationships between abstract symbols. Albeit non-significant, both cognitive subsets were consistent in our study in that they both showed an overall decline with decreasing $FiO_2$.

Similar to our data, no significant effect of acute hypoxia on cognition was seen in a previous study using a higher $FiO_2$ of 0.15 and 0.18 [11]. These authors used the Go/No-Go task with visual stimuli, similar to our MR task. Another study also showed no cognitive decline after exposing subjects to hypobaric hypoxia equivalent to 12,000 feet ($FiO_2 \approx 0.13$) [19]. Komiyama et al. used a $FiO_2$ of 0.12–0.13 and showed no effect on cognition with acute hypoxic exposure [20]. A recent study using a $FiO_2$ of 0.135 showed no effect on the Stroop test [21].

Contradictorily, a study using an acute low $FiO_2$ of 0.08 showed significant cognitive impairment using the King-Devick test [10]. This test is used for concussion detection and contrasts from BD and MR testing. Interestingly, this test requires subjects to read numbers aloud correctly, which in this study was done while subjects wore an aviator mask. For this reason, we decided not to use a cognitive test involving any verbal interaction. Furthermore, any subject with dyscalculia would be disadvantaged. A $FiO_2$ of 0.10 given for 90 minutes has also shown a decrease in cognition when using a battery of seven tests covering attention, memory,

and executive function domains [22]. In comparison, cognitive domains assessed in this study are similar to MR, but not BD. Additionally, our study took several minutes to 30 minutes for this study. Ochi et al. also have shown a decrease in executive function using various levels of $FiO_2$, the lowest being 0.105 [23]. A study investigating subjects on a high-altitude trek (5,160 m; $FiO_2 \approx 0.17$) has shown some decreases in executive function [24]. All these studies discussed above exposed subjects to hypoxia in a similar time frame compared to our study, albeit one [24]. A meta-analysis has shown a moderate inhibitory effect of hypoxia on cognition [1].

**Table 5 •** Compilation of several studies with $SpO_2$ values in decreasing numerical order with their cognitive effect and motor kinesthetic component

| Study and sample size | Lowest mean $SpO_2$ during testing (%) | Motor kinesthetic use | Cognitive function effect |
|---|---|---|---|
| Current study $n = 17$ | 90 | Yes | No |
| Ando et al., $n = 12$ (*11*) | 89 | None | No |
| Komiyama et al., $n = 13$ (*20*) | 88 | None | No |
| Legg et al., $n = 36$ (*19*) | 88 | None | No |
| Ochi et al., $n = 15$ (*21*) | 86 | None | No |
| Lefferts et al., $n = 18$ (*24*) | 80 | None | Mixed |
| Ochi et al., $n = 21$ (*23*) | 79 | None | Yes |
| Stepanek et al., $n = 25$ (*10*) | 76 | None | Yes |
| Turner et al., $n = 22$ (*22*) | 75 | None | Yes |

Although previous work has shown variations in cognitive deficit with the degree of hypoxia, it seems this clearly relates to $SpO_2$ levels. McMorris et al. compared cognitive deficit with $PaO_2$ showing no effect [1]. We have compiled data from the above studies, and they show decreases in cognitive function when $SpO_2 \leq 80\%$ (**Table 5**). We speculate that this could be a cut-off or threshold value, which needs to be reached before any cognitive deficit occurs. A meta-analysis could confirm this. Published data have shown a correlation between cognitive decline and low $SpO_2$ [23].

The majority of our HRV and cardiovascular data showed no statistical significance over the three gas interventions. However, the non-normalized HFnu value during MR was significantly lower across all tests in a dose-dependent manner. Comparatively, our MR and BD HR data showed a significant increase with lower $FiO_2$ with a dose effect. This is reinforced by Buchheit et al. who showed significant decreases in HF and increases in HR with acute hypoxia ($FiO_2 = 0.115$; [9]). HF modulation of an individual is primarily regulated through the vagus nerve and mirrors parasympathetic activity [8], which, when decreased, elevates the heart rate. Conversely, the SNS works antagonistically, as the activation results in an increased heart rate and myocardial contractility, therefore raising cardiac output and delivering more $O_2$ to tissues. The dose effect for HFnu suggests the PNS was suppressed as $FiO_2$ decreased, as has previously been reported in dogs [25]. There was a trend for LFnu to increase over all the MR tests, albeit non-significantly; therefore, it seems more likely the increase in HR was due to PNS inactivation. A reduction in HFnu activity has been found to be linked with decreased cognitive function which involves executive centers associated with the frontal lobe [8]. This links with our data, as both MR and BD scores had decreasing non-significant trends with increasing hypoxia.

For BD, TOI data over the three gas interventions did not show any significant change. Conversely, MR showed a significant dose effect decrease for TOI. Resting sea level TOI data from Davranche et al. were similar to our resting normoxia TOI data [15]. In this previous study, subjects taken to an altitude of 4.35 km ($FiO_2 \approx 0.12$) for four days have shown a significant fall of 11% in TOI [15], in comparison to our acute exposure resulting in a 4.4% decrease in MR. In another acute hypoxic study ($FiO_2 \approx 0.12–0.13$), no significant fall in cerebral oxygenation was noted [20]. A possible explanation for attenuated HFnu and TOI responses only occurring in MR but not in BD could be due to BD preceding MR, therefore resulting in MR having a longer exposure to hypoxia.

## 5. Limitations

A limitation of this study is a procedural learning effect (PLE) of the WASI-I. Most likely, the BD subset was more susceptible to PLE, compared to MR, as when the subject completed items in BD, they acquired the knowledge of how to construct certain designs. The knowledge of construction procedures learned from previous repeats may then have allowed subjects to form designs more quickly or accurately, thereby obtaining higher scores in subsequent repeats. Conversely, MR consisted of picking the correct matrices which completed the design shown, and hence having prior knowledge could be an advantage. However, as subjects were not informed of the correct answer for the matrix and the testing order was randomized, we believe PLE was negligible for MR. To improve the study, an increased time gap from 10 minutes to 2–3 days between each cognitive test at different $O_2$ interventions could provide sufficient time to de-familiarize with the WASI-I to nullify PLE. Due to possible loss of subject compliance, we opted for the same day testing. Another limitation was the sample size of 17, reducing the statistical power of the study, although several of the studies listed in **Table 5** had a sample size smaller than that of our current study. Separate data analysis based on gender or the order of experimentation was not conducted as this would further reduce the statistical power.

Five of the subjects were exposed to 0.12 $FiO_2$ first and were provided 10 minutes to recover. This limited recovery time could

have affected their cognitive abilities and their cardiovascular function when performing subsequent repeats. Mukandala et al. indicated that a hypoxic event lasting more than five minutes in the brain could affect cognitive performance [26]. This limitation can be circumvented by establishing a non-random process, where the order of $FiO_2$ interventions is 0.21, 0.165, and 0.12. However, possible physiological effects of repetitive testing would remain. Also, another limitation during the study was that subjects were not provided sufficient exposure time to the hypoxic gas. This could have been improved through waiting until a drop in $SpO_2$ was observed before testing.

Possible limitations of NIRS include variations in skin pigmentation [14], electrical noise, and skull thickness. However, an earlier study has negated these factors [27]. Another important point is that several studies used digitized cognitive testing [9, 19] which we did not use in our study; however, evidence shows no difference between traditional and digitized cognitive testing [28]. Lastly, we had a relatively young cohort of subjects, while most persons who are exposed to hypoxia, namely aircraft crew and mountaineers, are older.

Additionally, some tests could have been performed under conditions that would better imitate specific occupations under which might hypoxia occur, for example, pilots, and future work could include the use of a flight simulator.

## 6. Conclusions

This experiment demonstrated no significant effect of hypoxia on cognitive function in these subjects across the three gas interventions ($FiO_2$ of 0.21, 0.165, and 0.12). Although the WASI-I data were not significant, HR, one HRV, and one NIRS parameter did show significant changes. We suspect a $SpO_2$ cut-off around 80% is paramount in resulting in a cognitive deficit. This research is relevant to persons affected by environmental or pathological hypoxic conditions, in whom a normal cognitive function is critical. In light of the recent COVID-19 pandemic and subsequent rise in long COVID cases, the effect of hypoxia on cognition in these persons may require further investigation.

## Acknowledgments

The authors would like to thank Dr. Matt Roser, University of Plymouth for help and advice on the cognitive testing methodology. We also appreciate support with data collection from Natasha Carter, Rebecca Lang, and Laura Shute. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Faculty of Science and Engineering Research Ethics Committee, University of Plymouth. The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Funding

The authors report no conflict of interest and received no grant funding for this work.

## Author contributions

Author contribution is listed below:

1. Conception or design of the work (RR, MMFS)
2. Acquisition, analysis, or interpretation of data for the work (RR, MMFS)
3. Drafting of the work or revising it critically for important intellectual content (RR, MMFS)

We can confirm that all authors (RR, MMFS) approved the final version of the manuscript, 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 and that all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. Mr. Ravi is currently studying Medicine, and Dr. Subhan (ORCID id 0000-0002-1545-9995) is a Lecturer in Biomedical Science (Human Physiology).

## Conflicts of interest

The author(s) declare no conflict of interest.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Institutional review board statements

The study was approved by the Faculty of Science and Engineering Research Ethics Committee (28/10/2016) and conformed to the Declaration of Helsinki. The subjects were students of the University of Plymouth and were recruited by convenient sampling.

## Informed consent statement

All subjects gave informed and written consent before participation. The experimental protocol was explained to all subjects. Written informed consent has been obtained from the participant(s) to publish this paper.

## Sample availability

Not applicable.

## Additional information

Received: 2022-10-19

Accepted: 2023-01-02

Published: 2023-03-16

*Academia Biology* papers should be cited as *Academia Biology* 2023, ISSN pending, https://doi.org/10.20935/AcadBiol6070. The journal's official abbreviation is *Acad. Biol.*

## Copyright



## References

1. McMorris T, et al. Effect of acute hypoxia on cognition: a systematic review and meta-regression analysis. Neurosci Biobehav Rev. 2017;74:225–32.
2. Snyder HR, Miyake A, Hankin BL. Advancing understanding of executive function impairments and psychopathology: bridging the gap between clinical and cognitive approaches. Front Psychol. 2015;6. doi: 10.3389/fpsyg.2015.00328
3. Virués-Ortega J, Buela-Casal G, Garrido E, Alcázar B. Neuropsychological functioning associated with high-altitude exposure. Neuropsychol Rev. 2004;14(4):197–24.
4. Castor M, Borgvall J. The effects of hypoxic hypoxia on cognitive performance decay [Internet]. 2015 [cited 19/10/2022]. Available from: https://www.stralsakerhetsmyndigheten.se/en/publications/reports/safety-at-nuclear-power-plants/2015/201520/
5. Sutton JE, Buset M, Keller M. Navigation experience and mental representations of the environment: do pilots build better cognitive maps? PLoS One. 2014;9(3). doi: 10.1371/journal.pone.0090058
6. O'Driscoll, BR, Howard, LS, Davison. AGBTS guideline for emergency oxygen use in adult patients. Thorax. 2008;63:1–81.
7. Sharma S, Hashmi MF, Burns B. Alveolar gas equation. StatPearls: StatPearls Publishing; 2022. https://www.ncbi.nlm.nih.gov/books/NBK482268/
8. McCraty R, Shaffer F. Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. Glob Adv Health Med. 2015;4(1):45–61.
9. Buchheit M, et al. Effect of acute hypoxia on heart rate variability at rest and during exercise. Int J Sports Med. 2004;25(4):264–69.
10. Stepanek J, et al. Early detection of hypoxia-induced cognitive impairment using the King-Devick test. Aviat Space Environ Med. 2013;84(10):1017–22.
11. Ando S, et al. The effects of exercise under hypoxia on cognitive function. PLoS One. 2013;8(5). doi: 10.1371/journal.pone.0063630.
12. The Psychological Corporation. Wechsler abbreviated scale of intelligence. San Antonio: Harcourt Brace & Co.; 1999.
13. Pierson EE, Kilmer LM, Rothlisberg BA, McIntosh DE. Use of brief intelligence tests in the identification of giftedness. J Psychoedu Assess. 2012;30(1):10–24.
14. Scheeren TWL, Schober P, Schwarte LA. Monitoring tissue oxygenation by near infrared spectroscopy (NIRS): background and current applications. J Clin Monit Comput. 2012;26(4):279–87.
15. Davranche K, et al. Cognitive functions and cerebral oxygenation changes during acute and prolonged hypoxic exposure. Physiol Behav. 2016;164:189–97.
16. Sacha J. Interaction between heart rate and heart rate variability. Annals Noninvasive Electrocardiol. 2014;19(3):207–16.
17. Aldrete JA, Aldrete EL. Oxygen concentrations in commercial aircraft flights. South Med J. 1983;76:12–14.
18. Armstrong RA. When to use the Bonferroni correction. Ophthalmic Physiol Opt. 2014;34(5):502–8.
19. Legg SJ, et al. Effects of mild hypoxia in aviation on mood and complex cognition. Appl Ergon. 2016;53:357–63.
20. Komiyama T, et al. Cognitive function during exercise under severe hypoxia. Sci Rep. 2017;7(1):1–11.
21. Ochi G, et al. Neural basis for reduced executive performance with hypoxic exercise. Neuroimage. 2018a;171:75–83.
22. Turner CE, Barker-Collo SL, Connell CJW, Gant N. Acute hypoxic gas breathing severely impairs cognition and task learning in humans. Physiol Behav. 2015;142:104–10.
23. Ochi G, et al. Hypoxia-induced lowered executive function depends on arterial oxygen desaturation. J Physiol Sci. 2018b;68(6):847–53.
24. Lefferts WK, et al. Changes in cognitive function and latent processes of decision-making during incremental ascent to high altitude. Physiol Behav. 2019;201:139–45.
25. Potter EK, McCloskey DI. Effects of hypoxia on cardiac vagal efferent activity and on the action of the vagus nerve at the heart in the dog. J Auton Nerv Syst. 1986;17(4):325–9.
26. Mukandala G, Tynan R, Lanigan S, O'Connor JJ. The effects of hypoxia and inflammation on synaptic signaling in the CNS. Brain Sci. 2016;6(6):1–14.
27. Kenji Y, et al. Effects of hemoglobin concentration, skull thickness, and the area of the cerebrospinal fluid layer on near-infrared spectroscopy measurements. Anesthesiology. 2007;106(3):458–62.
28. Björngrim S, et al. Comparing traditional Sand digitized cognitive tests used in standard clinical evaluation – A study of the digital application Minnemera. Front Psychol. 2019;10. https://doi.org/10.3389/fpsyg.2019.02327