Title:

Cognitive performance is associated with cerebral oxygenation and peripheral oxygen saturation, but not plasma catecholamines, during graded normobaric hypoxia

Authors:

Thomas B. Williams[1], Jo Corbett[1], Terry McMorris[1,3], John S. Young[2], Matt Dicks[1], Soichi Ando[4], Richard C. Thelwell[1], Michael J. Tipton[1], Joseph T. Costello[1]

Affiliations:

1. Extreme Environments Laboratory, Department of Sport and Exercise Sciences, University of Portsmouth (UK)
2. School of Pharmacy and Biomedical Science, University of Portsmouth (UK)
3. Department of Sport and Exercise Sciences, University of Chichester (UK)
4. Graduate School of Informatics and Engineering, The University of Electro-Communication (Japan)

Corresponding Author:

Dr Joseph Costello

Extreme Environments Laboratory (EEL), Department of Sport and Exercise Science, 16 Spinnaker Building, Cambridge Road, University of Portsmouth, Portsmouth, PO1 2ER, UK

Email: Joe.costello@port.ac.uk

This is an Accepted Article that has been peer-reviewed and approved for publication in the Experimental Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an Accepted Article; doi: 10.1113/EP087647.

Running Title: Cognitive function during graded normobaric hypoxia.

Key words: catecholamines, cerebral oxygenation, cognition, simulated altitude, executive function

Total Number of Words: 6246

Total Number of References: 53

Funding: This research did not receive any funding.

Competing Interests: No competing interests declared.

Author contributions:

T.B.W., J.T.C., J.C., and T.M.: Conception, design, interpretation of data, drafting and revising the manuscript critically for important intellectual content. J.S.Y., M.D., S.A., R.C.T., and M.J.T.: Data acquisition, analysis, interpretation, drafting, and revising of the manuscript. All authors approved the final version of the manuscript and 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 are listed.

Acknowledgements:

We would like to thank the participants for volunteering for this study. We also wish to thank Danny White, Geoff Long and Harry Mayes for their technical assistance and Christopher Richards for his help with data collection.

New Findings:

What is the central question of this study?

What are the mechanisms responsible for the decline in cognitive performance following exposure to acute normobaric hypoxia?

What are the main findings and their importance?

We found that 1) performance of a complex central executive task (n-back) was reduced FiO_2 0.12; 2) there was a strong correlation between performance of the n-back task and reductions in SpO_2 and cerebral oxygenation; and 3) plasma adrenaline, noradrenaline, cortisol, and copeptin were not correlated with cognitive performance.

Abstract

It is well established that hypoxia impairs cognitive function; however, the physiological mechanisms responsible for these effects have received relatively little attention. This study examined the effects of graded reductions in fraction of inspired oxygen (FiO_2) on oxygen saturation (SpO_2), cerebral oxygenation, cardiorespiratory variables, activity of the sympathoadrenal system (adrenaline, noradrenaline) and hypothalamic-pituitary-adrenal axis (cortisol, copeptin), and cognitive performance. Twelve healthy males (mean [SD], age: 22 [4] yrs, height: 178 [5] cm, mass: 75 [9] kg, FEV_1/FVC ratio: 85 [5] %) completed a 4-task battery of cognitive tests to examine inhibition, selective attention (Eriksen Flanker), executive function (n-back) and simple and choice reaction time (Deary-Liewald). Tests were completed before and following 60 minutes of exposure to FiO_2 0.2093, 0.17, 0.145, and 0.12.

Following 60 minutes of exposure response accuracy in the n-back task was significantly reduced in FiO_2 0.12 compared to baseline (82 [9] vs. 93 [5] %; p < 0.001) and compared to all other conditions at the same time point (FiO_2 0.2093: 92 [3] %, FiO_2 0.17: 91 [6] %, FiO_2 0.145: 85 [10] %, FiO_2 12: 82 [9] %; all p < 0.05). The performance of the other tasks was maintained. Δ accuracy and Δ reaction time of the n-back task was correlated with both Δ SpO_2 (r (9) = 0.66; p < 0.001 and r (9) = - 0.36; p = 0.037 respectively) and Δ cerebral oxygenation (r (7) = 0.55; p < 0.001 and r (7) = - 0.38; p = 0.045 respectively). Plasma adrenaline, noradrenaline, cortisol and copeptin were not significantly elevated in any condition or correlated with any of the tests of cognitive performance. These findings suggest that reductions in peripheral oxygen saturation and cerebral oxygenation, and not increased activity of the sympathoadrenal system and hypothalamic-pituitary-adrenal axis, as previously speculated, are responsible for a decrease in cognitive performance during normobaric hypoxia.

Abbreviations

FiO_2: Fraction of inspired oxygen
SpO_2: Oxygen saturation
SAS: Sympathoadrenal system
HPA axis: Hypothalamic-pituitary-adrenal axis
PFC: Prefrontal cortex
NIRS: Near-infrared spectroscopy
RT: Reaction time
CRT: Choice reaction time
SRT: Simple reaction time
AMS: Acute mountain sickness
ECG: Electrocardiogram

f_R: Respiratory frequency

HHb: Deoxyhaemoglobin

O_2Hb: Oxyhaemoglobin

PaO_2: Partial pressure of oxygen

$P_{ET}CO_2$: Pressure of end tidal carbon dioxide

$P_{ET}O_2$: Pressure of end tidal oxygen

TSI: Tissue saturation index

$\dot{V}_E$: Minute ventilation

V_T: Tidal volume

1. Introduction

The brain requires a constant supply of oxygen in order to support the high rate of ATP production necessary to remain in an electrically active state (Ainslie, Hoiland, & Bailey, 2016; Bailey, Bärtsch, Knauth, & Baumgartner, 2009). Furthermore, as brain activity increases, for example during the performance of a cognitively demanding task, there is an associated rise in the metabolic demands of the neural tissues (Raichle & Gusnard, 2002). As such, a reduction in oxygen availability, as experienced at terrestrial high altitude, can have a detrimental effect on brain function, causing a decline in cognitive performance (Hoiland, Bain, Rieger, Bailey, & Ainslie, 2016).

Despite the growing volume of studies that have examined the effects of hypoxia on cognitive function, the large disparity in methodologies, heterogeneity of cognitive tests, and limited methodological designs (e.g. lack of a control group or normoxic comparison) have resulted in inconsistent findings (Petrassi, Hodkinson, Walters, & Gaydos, 2012; Taylor, Watkins, Marshall, Dascombe, & Foster, 2016; Virués-Ortega, Buela-Casal, Garrido, & Alcázar, 2004; Yan, 2014). Moreover, many of the previous investigations, particularly in aviation research (Petrassi et al., 2012), have focused on establishing an elevation at which cognitive performance decreases, rather than attempting to explore the associated physiological mechanism(s) responsible for a decrease in performance. There is also ambiguity surrounding the type of cognitive tasks that are affected. For example, previous reviews have suggested that decrements in performance were more prominent when completing complex central executive tasks (Petrassi et al., 2012; Taylor et al., 2016; Virués-Ortega et al., 2004; Yan, 2014). Given that more complex central executive tasks activate multiple regions of the brain (Nee, Wager, & Jondies, 2007; Owen, McMillan, Laird, &

Bullmore, 2005), these findings suggest that more demanding cognitive tasks may be more susceptible to hypoxia due to the necessity for greater neural activation. However, using meta-regression analysis, our group has recently shown that the detrimental effects of hypoxia were evident in both complex tasks requiring executive function and also in comparatively simple tasks (e.g. choice and simple reaction time tests) (Terry McMorris, Hale, Barwood, Costello, & Corbett, 2017). That said, our analysis, as with previous reviews, was limited by the number and quality of studies available for inclusion (22 studies comprising only 437 participants). Thus, although it is considered that cognitive performance markedly decreases as the severity of hypoxia increases, the physiological threshold at which impairment begins, the types of cognitive tasks affected and the mechanisms responsible require further investigation.

Recently, Ochi and colleagues demonstrated a decline in the performance of the Stroop task (i.e., a complex central executive task) was associated with decreased SpO_2 during graded normobaric hypoxia (Ochi et al., 2018). These findings indicate arterial oxygen desaturation as a potential physiological factor resulting in hypoxia-induced lowered central executive function. Unfortunately, this investigation did not measure cerebral oxygenation. Under hypoxia cerebral blood flow is increased to maintain cerebral oxygen delivery (Hoiland et al., 2016). However, cerebral blood flow is unevenly distributed, with greater blood flow directed towards regions of the brain with essential homeostatic roles (e.g. the cerebellum, hypothalamus, thalamus, basal ganglia and brainstem; regions of the brain concerned with cardiorespiratory control) (Binks, Cunningham, Adams, & Banzett, 2007; Willie et al., 2012). Using near infrared spectroscopy (NIRS) the prefrontal cortex (PFC), the primary region of the brain associated with greater activation during the performance of more complex,

central executive tasks (Arnsten & Li, 2005), has been shown to experience a reduction in oxygen saturation during hypoxic exposure (Davranche et al., 2016; Komiyama et al., 2017). Therefore, to elucidate the mechanism(s) responsible for cognitive decline under hypoxic conditions it is also important to investigate the role of cerebral oxygenation in addition to SpO_2.

Another important avenue of exploration that requires further investigation is the relationship between cognitive performance under hypoxia and alterations in catecholamines and stress hormones. Physical and psychological stress, leads to activation of the sympathoadrenal system (SAS) and increased activity of the hypothalamic-pituitary-adrenal (HPA) axis (Schoofs, Preuß, & Wolf, 2008). The initial response of the SAS is mediated via catecholamines, namely adrenaline and noradrenaline. The second, and slightly slower response, consists of activation of the HPA axis and leads to the release of glucocorticoids from the adrenal cortex (e.g. cortisol) (Schoofs et al., 2008). Following acute exposure to hypoxia, resting plasma and / or urinary catecholamines and stress hormones have been shown to significantly increase in comparison to normoxic concentrations (Kamimori et al., 2009; Panjwani, Thakur, Anand, Malhotra, & Banerjee, 2006). As mentioned, the PFC is the primary region of the brain activated during the performance of central executive tasks (Arnsten & Li, 2005). In addition, the performance of simple and choice reaction time type tasks are also associated with activation of the PFC, although they require considerably less neural activation (Barbas, 2000). However, the PFC is highly sensitive to its neurochemical environment and is therefore highly susceptible to stress (Arnsten, 2009). Therefore, these changes have important implications for cognitive performance. Normal function of the PFC is modulated via catecholamine neurotransmitters, in particular dopamine and noradrenaline

signalling in the PFC are critical modulators of prefrontal cognition (Arnsten & Li, 2005). Both dopamine and noradrenaline have an 'inverted U'-shaped influence on PFC function engaging different types of receptors with varying levels of release, such that both inadequate and excessive levels, may cause dysfunction (Arnsten, 2009; Berridge & Spencer, 2016; Terry McMorris, 2016). In addition, it is well established that glucocorticoid hormones released from the adrenal cortex during stress may also impair cognitive performance, with further evidence to suggest that noradrenaline and cortisol may have an additive effect on the decrease in central executive function (Elzinga & Roelofs, 2005). Plasma adrenaline has also been shown to be a predictor of prefrontal cognition, however, this is probably due to the relationship between secretion of adrenaline by the adrenal medulla and the release or noradrenaline in the central nervous system by the hypothalamus via the HPA axis (Terry McMorris, Swain, et al., 2006). Previous research has already examined the influence of alterations in catecholamines on cognitive performance during other forms of stress including sleep deprivation (Terry McMorris et al., 2007), exercise (T. McMorris, Collard, Corbett, Dicks, & Swain, 2008) and heat (Terry McMorris, Swain, et al., 2006), however, to date, there is no empirical evidence directly examining this relationship during hypoxia.

Whilst the aforementioned narrative (Petrassi et al., 2012; Taylor et al., 2016; Virués-Ortega et al., 2004; Yan, 2014) and systematic reviews (Terry McMorris et al., 2017) have provided an insight into this field, the substantial inter-individual variation in the response to hypoxia has made understanding the mechanism(s) by which cognition may be impaired considerably difficult. Therefore, the hypoxia-cognition relationship may be better understood if tested empirically and systematically, whereby participant's cognitive performance and physiological responses are measured

simultaneously during graded levels of hypoxia within a single study. Using a rigorous experimental design, the present study sought to examine simple and complex task performance during exposure to a range of mild-moderate normobaric hypoxic exposures (fraction of expired oxygen [FiO_2] 0.2093, 0.17, 0.145, 0.12) and the concurrent changes in cerebral oxygenation, cardiorespiratory physiology and peripheral biomarkers of SAS and HPA axis activity. We hypothesised that: 1) performance of less complex tasks (i.e. simple and choice reaction time) that require comparatively less neural activation of the PFC and other regions of the brain would be less likely to be reduced following hypoxia than more complex central executive tasks (i.e. Eriksen Flanker test of inhibition and selective attention and n-back) that require comparatively more neural activation of the PFC; and 2) complex task performance would be correlated with changes in SpO_2, cerebral oxygenation and plasma biomarkers associated with SAS and HPA axis activity.

2. Methods

2.1. Ethical approval and Study population

All experimental procedures adhered to the standards set by the latest revision of the declaration of Helsinki, except for registration in a database, and were approved by the Science Faculty Ethics Committee of The University of Portsmouth (project number 2017-025).

A convenience sample of 12 healthy male participants were recruited and participated in this study (mean [SD], age: 22 [4] yrs, height: 178 [5] cm, mass: 75 [9] kg FEV_1/FVC ratio: 85 [5] %. Due to known physiological changes during the menstrual cycle (blood volume, oxygen carrying capacity, ventilation, and body temperature alterations (Takano, 1984)) and the methodological design of the study

(4x conditions) we would not be able to categorically conclude that our findings were attributable to the response of hypoxia or the stage of menses in females; therefore females were not recruited. Following familiarisation with the laboratory and testing procedures, participants provided written, informed consent. Prior to experimentation, participants underwent thorough medical screening to assess for any contraindications to taking part. This included a 12-lead electrocardiogram (ECG), lung function examination and a full blood count. Participants were all non-smokers and were free of any known cardiovascular, respiratory or neurological disorders. All participants resided at < 500 m and had not spent time altitude for at least three months prior to commencement of the study, including travelling on commercial flight. Participants were instructed to refrain from any strenuous exercise, caffeine and alcohol in the 24 h preceding each visit to the laboratory. In addition, participants were requested to record their dietary intake for 24 h prior to their first visit and to replicate their eating habits for each visit thereafter.

2.2. Study design and Experimental procedures

The study employed a single-blind, randomised, controlled design. Participants were required to visit the laboratory on 6 occasions (health screening, familiarisation and 4 experimental trials). For each experimental trial participants were exposed, using a purpose-built environmental chamber (Sporting edge, Sherfield on Loddon, UK), to normobaric hypoxia for ~60 minutes at one of the following FiO_2 values: 0.2093 (sea-level), 0.17 (equivalent to ~1600 m), 0.145 (~3000 m), and 0.12 (~4500 m). Following extensive pilot work, an exposure time of 60 minutes was used as this time frame was sufficient for all cardiorespiratory variables to plateau. However, we acknowledge that a longer exposure could lead to AMS and mood disturbance and this may lead to a more pronounced decrease in cognitive performance (De Aquino

Lemos et al., 2012). Participants were blinded to the condition they were undertaking and completed an exit questionnaire once all trials were completed to establish whether they were able to correctly identify the conditions for each trial. These questionnaires revealed that the blinding was successful; none of the participants correctly identified the ordering of their conditions. The ambient temperature was maintained at 24 °C and the relative humidity was controlled at 50 % throughout (Komiyama et al., 2015; Sudo et al., 2017). Experimental trials were separated by a minimum of 48 h and conducted at the same time of day. During the exposure, if end tidal O_2 ($P_{ET}O_2$) or end tidal CO_2 ($P_{ET}CO_2$) fell below 45 mmHg and 25 mmHg respectively, for three consecutive breaths, or if SpO_2 went below 65 %, participants were given a supply of normoxic air and subsequently removed from the chamber.

For each trial, the participant was first instrumented outside of the chamber. They then entered the chamber and were connected to a supply or normoxic air (FiO_2 0.2093). The participant then rested in a semi-reclined position for approximately 10-minutes. The participant then completed the cognitive tests, questionnaires and had a venous blood sample taken (described in 2.3). The supply of normoxic air was then removed and the participant was exposed to the environment (*i.e.* FiO_2 of 0.2093, 0.17, 0.145, or 0.12) within the chamber. Following 60 minutes of exposure, during which the participant remained seated and resting, the cognitive tests and questionnaires were repeated, and another venous blood was sample taken. The participant then left the chamber.

2.3. Measures

2.3.1. Cognitive function

Cognitive function was assessed using a 4-task cognitive battery on a laptop computer (HP Pavilion, China). The battery was designed to examine the performance of both simple and complex cognitive performance. To reduce the occurrence of a learning effect, participants completed each of the tasks 8 times during a familiarisation session, as previously recommended (Taylor et al., 2016). Additional tasks were completed if participants did not meet the following criteria: a) reaction time (RT) variability for the last three trials was ≤ 5 %; b) mean reaction time < 700 ms for n-back and flanker, 300 Ms for simple reaction time (SRT), and 450 for choice reaction time (CRT); and c) response accuracy ≥ 90 %. In addition, participants also completed one further practice with each of the tests before the commencement of each experimental trial. Participants were instructed to respond as accurately and as quickly possible. Tasks were presented each time in the order listed below.

2.3.1.1. n-back: Central executive function was assessed using the 3-back version of the n-back number task (Jaeggi, Buschkuehl, Perrig, & Meier, 2010). Participants were presented with a series of letters and required to indicate using the keyboard when the current stimulus was the same as the one presented three trials previously. The task contained 30 trials with each stimulus presented for a maximum of 2000 ms, with a new stimulus presented every 2500 ms. If the participant answered correctly the letter turned green, and if incorrectly it turned red. Accuracy and mean reaction time for correct responses were recorded for analysis.

2.3.1.2. Eriksen flanker: Inhibition and selective attention (central executive function) were assessed using a modified version of the Eriksen Flanker task (Eriksen, 1995). Participants were presented with 5 letters above a fixation point and required to respond according to the central letter, ignoring the flanker letters. They were instructed to press the 'L' key when the letters 'B' or 'V' appeared and the 'A' key when the letters 'X' or 'C' appeared. The flanker letters could be the same as the target stimuli or representing the pressing of the same button (e.g. an 'X' flanked by 'C' = congruent) or different to the stimulus letter representing pressing of the opposite button (e.g. 'X' flanked by 'B' = incongruent). The interval between the disappearance of the display and the onset of the next was 500 ms. The task included 30 trials in total. Accuracy and mean reaction time for correct responses were recorded for analysis.

2.3.1.3. Simple and choice reaction time

SRT and CRT were measured using the Deary-Liewald Reaction Time Task (Deary, Liewald, & Nissan, 2011). For SRT, a white square set against a blue background was positioned in the centre of the screen. When a black cross appeared within that square the participant had to respond by pressing the space bar as quickly as possible. For CRT, four white squares set against a blue background were positioned in a horizontal line across the middle of the computer screen. When a black cross appeared in one of the squares, the participant was required to press a corresponding key on the keyboard that was in alignment to the position of the squares on the screen. In both tasks, the cross remained on the screen until a button was pressed. The time interval between the response and when the next cross appeared ranged between 100 and 300 ms and was randomised within these

boundaries. Participants were required to complete 20 trials for each of the tasks. Mean reaction time was recorded for analysis, with accuracy also recorded for CRT.

2.4 Physiological measures

2.4.1 Cerebral oxygenation

Cerebral oxygenation was evaluated using the near-infrared spectroscopy (NIRS) technique as previously described (Ando et al., 2013). The NIRS device evaluates the amount of infrared light that effectively traverses an investigated tissue from a transmitter to a receiver. In doing so, the device can detect changes in oxygen-dependant light absorption by oxy- and deoxy-haemoglobin (Pinti et al., 2018). The NIRS device (Portalite, Artinis Medical, The Netherlands) was used to measure oxygenation of the PFC at wavelengths between 760 and 850 nm. The Portalite was attached to the surface of the left PFC between Fp1 and F3 (international EEG 10-20 system). The measurement site was cleaned with an alcohol wipe and then covered with a transparent film prior to placement of the device. To protect from light interference the device was shielded using a black bandana (Kovalenko, Roskosky, Freedman, & Shuler, 2015). Measurements included an age-dependant differential path-length factor according to manufacturer's guidelines (Duncan et al., 1996). At the beginning of each trial, a resting baseline was established during the initial rest period. All NIRS data were then expressed as changes from baseline (Δ) (Komiyama et al., 2017). The change in oxyhaemoglobin (Δ [O_2Hb]), deoxyhaemoglobin (Δ [HHb]), total haemoglobin (Δ [tHb]), and tissue saturation index (Δ [O2Hb] / Δ [tHb] × 100 %) from baseline were then calculated. In addition, skin blood flow at the forehead was also measured using laser Doppler flowmetry (VMS-LDF2, Moor Instruments, Axminster, UK). These data were used to examine the influence that

hypoxia-induced reductions in skin blood flow may have had on the NIRS measurements (Sudo et al., 2017).

2.4.2 Cardiorespiratory variables

Minute ventilation ($\dot{V}_E$) respiratory frequency (f_R), tidal volume (V_T), and end-tidal pressure of CO_2 ($P_{ET}CO_2$) and O_2 ($P_{ET}O_2$) were measured breath-by-breath using a metabolic cart (Quark CPET, Cosmed, Rome, Italy) as previously described (Neal et al., 2017). Peripheral oxygen saturation (SpO_2) was measured using pulse oximetry on the index finger of the right hand (Nonin 7500, US). Heart rate (HR) was monitored via a 3-lead electrocardiogram (HME, Lifepulse, England). HR and SpO_2 were continually recorded using an analogue to digital data acquisition system (PowerLab 16sp, AD instruments, Castle Hill, Australia).

2.4.3. Blood collection and analysis

Blood was collected via venepuncture from the antecubital fossa at baseline and following 60 minutes of exposure once cognitive tests had been completed. The sample was immediately dispensed into a potassium ethylene diamine tetracetic acid vacutainer and placed on ice. The samples were then centrifuged at 4 °C for 20 minutes at 2500g (Haraeus, Multifuge 3SR Plus, Thermo Electron Scientific instruments, US). The plasma was then aliquoted and stored at -85 °C for later analysis. Commercially available enzyme-linked immunosorbent assays were then used to measure the concentrations of cortisol (Calbiotech, Spring Valley, California, USA), copeptin (Cloud-Clone Corp, Houston, Texas, USA) adrenaline and noradrenaline (both Novusbio, Abingdon, UK). Manufacturer guidelines were followed, and repeated freeze thaw cycles were minimised. Intra-assay coefficient of variation was 5.2 %, 17.0 %, 3.6 % and 4.1 % for cortisol, copeptin, adrenaline and

noradrenaline respectively. Minimum detectable plasma concentrations were 20 $ng.mL^{-1}$, 12.35 $pg.mL^{-1}$, 10 $pg.mL^{-1}$ and 36 $pg.mL^{-1}$ for cortisol, copeptin, adrenaline and noradrenaline respectively.

2.5. Subjective outcome measures

Acute mountain sickness (AMS) (Lake Louise Score) (Roach et al., 2018), dyspnoea (Modified Borg dyspnoea scale, 0, 'Nothing at all' to 10, 'Shortness of breath so severe you need to stop') (Wilson & Jones, 1989), and mood disturbance (POMS) (Curran, Andrykowski, & Studts, 1995) were self-reported at baseline and following 60 min of exposure.

2.6 Statistical analysis

Physiological data (NIRS and cardiorespiratory measures) were collected for the duration of the trials, however, data were only analysed for the period in which the participants were completing the cognitive tests. The distribution of data was assessed using descriptive methods (skewness, outliers, and distribution plots) and inferential statistics (Shapiro-Wilk test). Main and interaction effects for the physiological data (HR, SpO_2, O_2Hb, HHb, tHb, TSI, $\dot{V}_E$, V_T, f_R, $P_{ET}CO_2$, $P_{ET}O_2$) and subjective outcome measures (AMS, dyspnoea, mood disturbance) were analysed using a two-way (environment [FiO2: 0.2093, 0.17, 0.145, 0.12] x time [baseline, 60 minutes of exposure]) repeated measures analysis of variance (ANOVA). Cognitive task performance (RT and accuracy) were assessed using the same method, as were adrenaline, noradrenaline, cortisol and copeptin. Where sphericity was violated, degrees of freedom were corrected using the Greenhouse-Geisser method. Where appropriate, post-hoc analysis was performed using the least significant difference (LSD) method. The test of interest for each variable was the significance of the

interaction. Within-participant correlation coefficients were computed for correlations between $\dot{V}_E$, V_T, f_R, $P_{ET}CO_2$,HR, Δ SpO_2, Δ TSI, Δ cortisol, Δ copeptin, Δ adrenaline, Δ noradrenaline, mood disturbance, dyspnoea, AMS and cognitive task performance (Δ RT and Δ accuracy) using the method described by Bland and Altman [34]. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences), version 24.0 (SPSS Inc, Chicago, IL, USA). Data are presented as mean [SD] unless otherwise stated. Statistical significance was accepted at $p < 0.05$.

3. Results

One participant was removed from the chamber in FiO_2 0.12 ($P_{ET}O_2$ fell below 45 mmHg). Therefore, the following analyses are for the 11 participants that successfully completed all 4 experimental trials.

3.1. Cognitive function

Performance for each of the cognitive tasks are displayed in Table 1. Individual responses to the tasks (Δ RT and Δ accuracy) are shown in Figure 1. Analysis revealed no significant changes in RT for any of the tasks (all $p > 0.05$). Accuracy was maintained in each of the tasks with the exception of the n-back task, where there was a significant interaction effect ($F_{(3,30)} = 9.157$; $p < 0.001$). Following 60 minutes of exposure accuracy was significantly lower in FiO_2 0.12 compared to baseline ($p < 0.001$) and was significantly reduced compared to FiO_2 0.2093 ($p = 0.004$) and FiO_2 0.17 ($p = 0.002$) at the same time point. Accuracy was also significantly reduced in FiO_2 0.145 following 60 minutes of exposure compared to FiO_2 0.2093 ($p = 0.049$), however, it was not significantly different to baseline ($p > 0.05$).

3.2. Physiological measures

Participants commenced each of the experimental trials from a resting baseline, with no significant differences between trials (all $p > 0.05$, Table 2).

3.2.1. Cerebral oxygenation

Due to equipment malfunction the following NIRS analyses are for 9 participants only (Figure 2B-E). There was a significant interaction effect for Δ O_2Hb ($F_{(3,24)} = 7.304$; $p = 0.001$), Δ HHb ($F_{(3,24)} = 43.360$; $p < 0.001$) and Δ TSI ($F_{(3, 24)} = 12.810$; $p < 0.001$). In each condition, with the exception of FiO_2 0.2093, there was a significant reduction in Δ O_2Hb (all $p < 0.05$) and a reciprocal increase in Δ HHb compared to baseline (all $p < 0.05$). Following 60 minutes of exposure, Δ O_2Hb was significantly lower and Δ HHb was significantly higher with each reduction in FiO_2 (all $p < 0.05$). Similarly, Δ TSI was significantly reduced following 60 minutes of exposure compared to baseline in all conditions except for FiO_2 0.2093 (all $p < 0.05$). In FiO_2 0.12, Δ TSI was significantly lower than each of the other conditions following 60 minutes of exposure (all $p < 0.05$) There was no significant interaction effect for Δ tHb ($p = 0.055$). The correlation between Δ skin blood flow and Δ O_2Hb was not significant ($r(7) = -0.23$, $p = 0.237$).

3.2.2. Cardiorespiratory response

There were no significant changes in $\dot{V}_E$, V_T or f_R across conditions (all $p > 0.05$; Table 2). Compared to baseline, both $P_{ET}CO_2$ and $P_{ET}O_2$ were significantly reduced following 60 minutes of exposure in all conditions with the exception of FiO_2 0.2093 (all $p < 0.05$). As expected, there was a significant reduction in $P_{ET}O_2$ with each reduction FiO_2 (FiO_2 0.2093 > 0.17 > 0.145 > 0.12; all $p < 0.05$).

A significant interaction effect for SpO_2 was observed ($F_{(3,30)}$ = 107.902; p < 0.001; Figure 2A). There was a significant reduction in SpO_2 in all conditions following 60 minutes of exposure compared to baseline, with the exception of FiO_2 0.2093 (all p < 0.05). As designed, there was a concomitant decrease in SpO_2 with each reduction in FiO_2 (0.2093 > 0.17 > 0.145 > 0.12; all p < 0.05). A significant interaction was detected for HR ($F_{(3,30)}$ = 8.640; p < 0.001). Compared to baseline, there was a significant reduction in HR in FiO_2 0.2093 (p < 0.001) and a significant increase in FiO_2 0.12 (p = 0.023). Following 60 minutes of exposure, HR was significantly higher in both FiO_2 0.145 and 0.12 compared to 0.17 and 0.2093 (p < 0.05).

3.3. Biomarkers

There were no between environment differences observed for any of the biomarkers following 60 minutes of exposure (all p < 0.05). There were also no significant changes from baseline for any of these measures (Figure 3A-D).

3.4. Subjective outcome measures

The questionnaire data indicated no significant change in mood or dyspnoea. In addition, whilst some of the participants reported various symptoms of AMS (headache, feeling tired), none were classified as having AMS according to the Lake Louise AMS diagnosis criteria (Table 2).

3.5. Correlational analysis

Correlational analysis revealed a strong, positive correlation between Δ accuracy in the n-back task and both Δ SpO_2 (r (9) = 0.66; p < 0.001; figure 3B) and Δ TSI (r (7) = 0.55; p < 0.001; Figure 4D). We also observed a moderate negative correlation between Δ reaction time for the n-back task and both Δ SpO_2 (r (9) = -0.36; p =

0.037; Figure 4A) and Δ TSI (r (7) = - 0.38; p = 0.045; figure 4C). There was also a very strong, positive correlation between Δ TSI and Δ SpO_2 (r (7) = 0.77; p < 0.001). No other significant correlations were observed.

4. Discussion

This study is the first to systematically examine the relationship between cognitive performance and cerebral oxygenation, peripheral oxygen saturation, sympathoadrenal system and hypothalamic-pituitary-adrenal axis activity during exposure to graded mild- to moderate- levels of normobaric hypoxia. The principal novel findings from this study are: 1) accuracy on the n-back task (indicative of central executive function) was reduced at FiO_2 0.12; 2) central executive function, assessed using accuracy and reaction time on the n-back task, was significantly correlated with reductions in SpO_2 and cerebral oxygenation; and 3) peripheral levels of adrenaline, noradrenaline, cortisol and copeptin were *not* significantly elevated and were *not* correlated with the cognitive task performance assessed. Collectively, these findings suggest that central executive performance on complex cognitive tasks (n-back) decreases during rest at a simulated altitude equivalent to ~4500 m. This decrease in performance is associated with reductions in peripheral and cerebral oxygenation, but not sympathoadrenal system or hypothalamic-pituitary-adrenal axis activity as previously speculated in the literature.

In partial agreement with our first hypothesis, we observed maintained performance in SRT and CRT and a decline in performance of the n-back task (Table 1; Figure 1). However, in contrast to our hypothesis, we found no decrements in the performance of the flanker task. Firstly, it is unsurprising that we only observed a significant decrement in performance in the FiO_2 0.12 condition. Whilst several studies have

reported a decrease in cognitive performance at altitudes (or simulated altitudes) between 2000-3500 m (Terry McMorris et al., 2017; Petrassi et al., 2012), decrements in performance are more pronounced once elevation exceeds 4000 m. Thus, despite a significant reduction in cerebral oxygenation in each of the hypoxic conditions, it was not until desaturation reached ~5% that cognitive performance was significantly reduced.

Despite our recent meta-analysis (McMorris et al., 2017) suggesting that both simple and complex task performance are reduced following hypoxic exposure, we felt that due to the limited availability and variable quality of evidence on the topic, examination of both simple and complex task performance was required. Our hypothesis that simple task performance (SRT and CRT) would be less likely to be reduced following hypoxia than complex task performance (n-back and Eriksen flanker) was therefore based on the reported dysfunction of the PFC due to alterations in its neurochemical environment following psychophysiological stress (Arnsten, 2009; Arnsten & Li, 2005; Berridge & Spencer, 2016), and the requirement of several other brain regions that may also be susceptible to hypoxia. An interesting finding to emerge from the current study was that although a decrease in n-back performance was observed, performance of the flanker task was maintained. Whilst both the n-back and flanker tasks are considered to be complex tasks that place a load on central executive function, the tasks are considerably different, and as such, may also therefore require activation of different regions of the brain. A meta-analysis of functional neuroimaging studies focusing on non-verbal n-back tasks (Owen, McMillan, Laird, & Bullmore, 2005) similar to that used in the current study, revealed brain activation in six key frontal and parietal regions, namely the right dorsolateral prefrontal, lateral premotor and posterior parietal cortex, a set of regions

that have been described as the spatial attention network. However, a meta-analysis of flanker studies observed brain activation in the right dorsolateral prefrontal cortex with additional activation in the right insula (Nee, Wager, & Jondies, 2007). Thus, in comparison to the flanker, the n-back appears to require activation of several different brain regions of which may be more susceptible to increased levels of hypoxia, however this proposal requires further investigation.

Although we did not measure the central executive (attention) demand of either task, it may be that the demands for the 3-back test are greater than those for the flanker. Scharinger et al. (Scharinger, Soutschek, & Schubert, 2015), using a combined n-back/flanker task, examined the effect of increased demand on the n-back factor of the task on performance of the flanker factor. They found an underadditive interference effect, i.e. facilitation of performance on the flanker factor when the n-back factor increased from 0- and 1-back to 2-back. This was the case for behavioural, electroencephalograph and pupillometry data. The authors interpreted this as showing that the activation of networks common to both tasks was so great during the 2-back test that the flanker task required no extra resources unlike in the 0-back and 1-back conditions. This does suggest that 3-back may require a greater attention demand than the flanker task.

In a study that investigated cerebral oxygenation during hypoxia, Subudhi et al. (2009) reported that the extent of deoxygenation was greatest in the prefrontal areas, however, patterns of deoxygenation were similar across multiple regions of the brain (i.e. prefrontal, premotor, and motor cortices (Subudhi, Miramon, Granger, & Roach, 2009). Thus, although we only measured oxygenation of the PFC, it is reasonable to suggest that hypoxia resulted in a similar reduction in oxygenation across the multiple brain regions activated during the n-back task. Therefore, we

speculate that the reduction in n-back performance was caused by (i) reduced oxygenation of the PFC and the other regions of the brain associated with n-back performance, and (ii) also, or in isolation, the addition of the hypoxic stressor altered the interaction between these regions. This may potentially explain the lack of relationship between the less complex tasks and both SpO_2 and cerebral oxygenation. Future research is warranted to explore these proposed physiological mechanism(s) as the current research suggests that the effects of hypoxia on cognitive performance is related to the degree of neural activation required to complete the task.

In partial agreement with our second hypothesis, n-back performance (RT and accuracy) was significantly correlated with both Δ SpO_2 and Δ TSI (Figure 3). These findings support those of Ochi et al., (2018) who reported that SpO_2 was associated with performance of the Stroop task in hypoxic conditions similar to those used in this study (FiO_2 = 0.165, 0.135, 0.105; r = -0.293, $p < 0.01$). Our data further extends the findings of Ochi et al., (2018) by providing the first experimental evidence, using a rigorous, within subject design, that n-back performance (a task requiring executive function) is associated with both peripheral arterial oxygen desaturation and oxygenation of the PFC. Oxygenation of the PFC is also affected by cerebral vasoconstriction caused by hypoxia-induced hyperventilation (Ogoh et al., 2014; Steinback & Poulin, 2008). Although we observed a significant alteration in $P_{ET}CO_2$, the magnitude of change was small (3 mmHg). Furthermore, there was also no significant correlation between $P_{ET}CO_2$ and TSI. In contrast, we observed a very strong positive correlation between SpO_2 and TSI ($r(7) = 0.77$; $p < 0.001$), suggesting that reduced arterial oxygenation had a greater role in deoxygenation of the PFC than hyperventilation-induced restriction of cerebral blood flow.

Furthermore, it is also important to note that we observed no relationship between Δ skin blood flow and Δ O_2Hb (p = 0.237) suggesting the NIRS device was providing a measure that accurately reflected changes in cerebral oxygenation and not skin blood flow.

Despite observing a considerable reduction in both SpO_2 and cerebral oxygenation we found no significant alterations in sympathoadrenal system or hypothalamic-pituitary-adrenal axis activity. Adrenaline, noradrenaline, cortisol, and copeptin values were within the expected range for normal resting values following 60 minutes of exposure and no relationship between any of the biomarkers and cognitive performance was observed. These findings support those of an earlier review that found only trivial changes in plasma catecholamines following hypoxic exposure (Rostrup, 1998). The minimal increases in cortisol, the most widely accepted measure of HPA axis activity (Costello et al., 2018; Morgan et al., 2000), $\dot{V}_E$, and heart rate suggest that the participants experienced only minor levels of physiological strain even in the most severe hypoxic exposure (Table 2). It is of great interest therefore that we still observed a significant decrease in cognitive performance on the n-back task. Although our findings suggest there was not an increase in noradrenaline, it is possible that as catecholamines do not cross the blood brain barrier, that this was not reflective of what was occurring in the brain (Terry McMorris, Harris, et al., 2006). However, we included the measurement of plasma concentrations as circulating adrenaline and noradrenaline activate β-adrenoceptors on the vagus nerve, which feeds back to the nucleus of the solitary tract and activates catecholaminergic neurons in the medulla and pons (Terry McMorris, 2016). These initiate the synthesis and release of noradrenaline from the locus coeruleus which projects to the PFC and indeed throughout the brain (Terry

McMorris, 2016). In addition, the turnover of catecholamines is altered in response to hypoxia due to the requirement of oxygen during synthesis, release and metabolism (Gibson, Pulsinelli, Blass, & Duffy, 1981). It is therefore plausible that although plasma noradrenaline concentration was unchanged, its turnover may have been. This may be indicative of a similar response in the brain. Furthermore, cortisol does cross the blood brain barrier (Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007) and so peripheral concentrations may be indicative of central levels. That cortisol also showed no significant differences between conditions supports the claim that hypoxia disrupts neurochemical turnover in the brain (Gibson et al., 1981). Nevertheless, future studies that measure changes in plasma concentrations of metabolites of noradrenaline and dopamine such as 3,4-methoxyhydroxyphenylglycol and homovanillic acid, which do cross the blood brain barrier may provide greater understanding of the mechanistic processes underpinning cognitive performance.

Further to the future directions considered thus far in the discussion, the experimental design used in the present study and associated findings have created interesting hypotheses that warrant further investigation. Firstly, whilst this study has expanded on the work of Ochi et al. (2018) by including a measure of cerebral oxygenation and plasma biomarkers associated with SAS and HPA axis activity, we did not measure cerebral blood flow. This is an important variable which should be considered in future investigations (Ogoh et al., 2014). A potential limitation of this study may have been that participants only rested for 10 minutes prior to the start of the experiment. Consequently, ventilation may have been elevated at the beginning of the experiment and therefore explain why we observed no changes in ventilation after 60 minutes of exposure. This appears to be further confirmed by the decrease

in heart rate we observed in the FiO_2 0.2093 condition. Future studies may want to ensure a greater rest time is given prior to commencement. In order to exclude any confounding factors brought about by exercise, the current study was conducted whilst participants were at rest, however, it is well accepted that cognition may be improved during moderate intensity exercise (Chang, Labban, Gapin, & Etnier, 2012; Terry McMorris, 2016). Furthermore, there is emerging evidence to suggest that the beneficial effects of exercise may outweigh the negative effects of hypoxia, dependent upon the severity of hypoxia and the intensity of the exercise (Komiyama et al., 2017). With a high proportion of activities taking place at altitude involving exercise (e.g. skiing, military operations, mountain rescue) future studies, using a similar robust methodological construct, should examine changes in cognitive performance when exercising. Also, hypoxia is seldom experienced in isolation (M. Tipton, 2012; M. J. Tipton, 2016) and is often encountered alongside various other stressors such as fatigue, dehydration, sleep deprivation, or other environmental conditions such as the cold or the heat. As this is one of the first studies attempting to delineate the physiological mechanism(s) responsible for cognitive decline in hypoxia, further research is required to examine the impact of combining these additional stressors with hypoxia. It is also important to note that the rigorous health screening undertaken by participants in this study meant that all participants were in excellent health and as such, this may not have been a true representation of the average population. This may also explain why previous studies with no such pre-screening have experienced greater decrements in performance at lower levels of hypoxia. Finally, considering the influence of sex on cerebral function (Robison, Gannon, Salinero, & Zuloaga, 2019), it is necessary for future studies to confirm these findings in females.

In conclusion, hypoxia-induced decreases in central executive performance are associated with reductions in both SpO_2 and cerebral oxygenation, but not plasma adrenaline, noradrenaline, cortisol, or copeptin. Nevertheless, the substantial majority of the inter-individual variance in cognitive performance following exposure to hypoxia remains unaccounted for and future studies should seek to increase understanding of the physiological factors contributing to a decrease in central executive performance.

References

Ainslie, P. N., Hoiland, R. L., & Bailey, D. M. (2016). Lessons from the laboratory; integrated regulation of cerebral blood flow during hypoxia. *Experimental Physiology*, *101*(9), 1160–1166. http://doi.org/10.1113/EP085671

Ando, S., Hatamoto, Y., Sudo, M., Kiyonaga, A., Tanaka, H., & Higaki, Y. (2013). The Effects of Exercise Under Hypoxia on Cognitive Function. *PLoS ONE*, *8*(5). http://doi.org/10.1371/journal.pone.0063630

Arnsten, A. F. T. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Reviews Neuroscience*, *10*(6), 410–422. http://doi.org/10.1038/nrn2648

Arnsten, A. F. T., & Li, B. M. (2005). Neurobiology of executive functions: Catecholamine influences on prefrontal cortical functions. *Biological Psychiatry*, *57*(11), 1377–1384. http://doi.org/10.1016/j.biopsych.2004.08.019

Bailey, D. M., Bärtsch, P., Knauth, M., & Baumgartner, R. W. (2009). Emerging concepts in acute mountain sickness and high-altitude cerebral edema: From the molecular to the morphological. *Cellular and Molecular Life Sciences*, *66*(22), 3583–3594. http://doi.org/10.1007/s00018-009-0145-9

Barbas, H. (2000). Connections underlying the synthesis of cognition, memory, and emotion in primate prefrontal cortices. *Brain Research Bulletin*, *52*, 319–330. http://doi.org/10.1016/j.concog.2017.09.008

Berridge, C. W., & Spencer, R. C. (2016). Differential cognitive actions of norepinephrine a2 and a1 receptor signaling in the prefrontal cortex. *Brain Research*, *1641*, 189–196. http://doi.org/10.1016/j.brainres.2015.11.024

Binks, A. P., Cunningham, V. J., Adams, L., & Banzett, R. B. (2007). Gray matter blood flow change is unevenly distributed during moderate isocapnic hypoxia in humans. *Journal of Applied Physiology*, *104*(1), 212–217. http://doi.org/10.1152/japplphysiol.00069.2007

Chang, Y. K., Labban, J. D., Gapin, J. I., & Etnier, J. L. (2012). The effects of acute exercise on cognitive performance: A meta-analysis. *Brain Research*, *1453*(250), 87–101. http://doi.org/10.1016/j.brainres.2012.02.068

Costello, J. T., Rendell, R. A., Furber, M., Massey, H. C., Tipton, M. J., Young, J. S., & Corbett, J. (2018). Effects of acute or chronic heat exposure, exercise and dehydration on plasma cortisol, IL-6 and CRP levels in trained males. *Cytokine*, *110*(September 2017), 277–283. http://doi.org/10.1016/j.cyto.2018.01.018

Curran, S. L., Andrykowski, M. A., & Studts, J. L. (1995). Short form of the Profile of Mood States (POMS-SF): Psychometric information. *Psychological Assessment*, *7*(1), 80–83. http://doi.org/10.1037/1040-3590.7.1.80

Davranche, K., Casini, L., Arnal, P. J., Rupp, T., Perrey, S., & Verges, S. (2016). Cognitive functions and cerebral oxygenation changes during acute and prolonged hypoxic exposure. *Physiology and Behavior*, *164*, 189–197. http://doi.org/10.1016/j.physbeh.2016.06.001

De Aquino Lemos, V., Antunes, H. K. M., Dos Santos, R. V. T., Lira, F. S., Tufik, S., & De Mello, M. T. (2012). High altitude exposure impairs sleep patterns, mood, and cognitive functions. *Psychophysiology*, *49*(9), 1298–1306. http://doi.org/10.1111/j.1469-8986.2012.01411.x

Deary, I. J., Liewald, D., & Nissan, J. (2011). A free, easy-to-use, computer-based

simple and four-choice reaction time programme: The Deary-Liewald reaction time task. *Behavior Research Methods*, *43*(1), 258–268. http://doi.org/10.3758/s13428-010-0024-1

Duncan, A., Meek, J. H., Clemence, M., Elwell, C. E., Fallon, P., Tyszczuk, L., … Delpy, D. T. (1996). Measurement of Cranial Optical Path Length as a Function of Age Using Phase Resolved Near Infrared Spectroscopy. *Pediatric Research*, *39*, 889. Retrieved from http://dx.doi.org/10.1203/00006450-199605000-00025

Elzinga, B. M., & Roelofs, K. (2005). Cortisol-Induced Impairments of Working Memory Require Acute Sympathetic Activation, *119*(1), 98–103. http://doi.org/10.1037/0735-7044.119.1.98

Eriksen, C. W. (1995). The Flankers Task and Response Competition: A Useful Tool for Investigating a Variety of Cognitive Problems. *Visual Cognition*, *2*(2–3), 101–118. http://doi.org/10.1080/13506289508401726

Gibson, G. E., Pulsinelli, W., Blass, J. P., & Duffy, T. E. (1981). Brain dysfunction in mild to moderate hypoxia. *The American Journal of Medicine*, *70*(6), 1247–1254. http://doi.org/10.1016/0002-9343(81)90834-2

Hoiland, R. L., Bain, A. R., Rieger, M. G., Bailey, D. M., & Ainslie, P. N. (2016). Hypoxemia, oxygen content, and the regulation of cerebral blood flow. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *310*(5), R398–R413. http://doi.org/10.1152/ajpregu.00270.2015

Jaeggi, S. M., Buschkuehl, M., Perrig, W. J., & Meier, B. (2010). The concurrent validity of the N-back task as a working memory measure. *Memory*, *18*(4), 394–412. http://doi.org/10.1080/09658211003702171

Kamimori, G. H., Ryan, E. J., Otterstetter, R., Barkley, J. E., Glickman, E. L., & Davis, H. Q. (2009). Catecholamine levels in hypoxia-Induced acute mountain sickness. *Aviation Space and Environmental Medicine*, *80*(4), 376–380. http://doi.org/10.3357/ASEM.2336.2009

Komiyama, T., Katayama, K., Sudo, M., Ishida, K., Higaki, Y., & Ando, S. (2017). Cognitive function during exercise under severe hypoxia. *Scientific Reports*, *7*(1), 1–11. http://doi.org/10.1038/s41598-017-10332-y

Komiyama, T., Sudo, M., Higaki, Y., Kiyonaga, A., Tanaka, H., & Ando, S. (2015). Does moderate hypoxia alter working memory and executive function during prolonged exercise? *Physiology and Behavior*, *139*, 290–296. http://doi.org/10.1016/j.physbeh.2014.11.057

Kovalenko, B., Roskosky, M., Freedman, B. A., & Shuler, M. S. (2015). Effect of Ambient Light on Near Infrared Spectroscopy. *Trauma & Treatment*, *2015*(November), 911–916. http://doi.org/10.4236/ajac.2015.612086

Levine, A., Zagoory-Sharon, O., Feldman, R., Lewis, J. G., & Weller, A. (2007). Measuring cortisol in human psychobiological studies. *Physiology and Behavior*, *90*(1), 43–53. http://doi.org/10.1016/j.physbeh.2006.08.025

McMorris, T. (2016). Developing the catecholamines hypothesis for the acute exercise-cognition interaction in humans: Lessons from animal studies. *Physiology and Behavior*, *165*, 291–299. http://doi.org/10.1016/j.physbeh.2016.08.011

McMorris, T., Collard, K., Corbett, J., Dicks, M., & Swain, J. P. (2008). A test of the catecholamines hypothesis for an acute exercise-cognition interaction.

Pharmacology Biochemistry and Behavior, *89*(1), 106–115. http://doi.org/10.1016/j.pbb.2007.11.007

McMorris, T., Hale, B. J., Barwood, M., Costello, J., & Corbett, J. (2017). Effect of acute hypoxia on cognition: A systematic review and meta-regression analysis. *Neuroscience and Biobehavioral Reviews*, *74*, 225–232. http://doi.org/10.1016/j.neubiorev.2017.01.019

McMorris, T., Harris, R. C., Howard, A. N., Langridge, G., Hall, B., Corbett, J., … Hodgson, C. (2007). Creatine supplementation, sleep deprivation, cortisol, melatonin and behavior. *Physiology and Behavior*, *90*(1), 21–28. http://doi.org/10.1016/j.physbeh.2006.08.024

McMorris, T., Harris, R. C., Swain, J., Corbett, J., Collard, K., Dyson, R. J., … Draper, N. (2006). Effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, mood state, and plasma concentrations of catecholamines and cortisol. *Psychopharmacology*, *185*(1), 93–103. http://doi.org/10.1007/s00213-005-0269-z

McMorris, T., Swain, J., Smith, M., Corbett, J., Delves, S., Sale, C., … Potter, J. (2006). Heat stress, plasma concentrations of adrenaline, noradrenaline, 5-hydroxytryptamine and cortisol, mood state and cognitive performance. *International Journal of Psychophysiology*, *61*(2), 204–215. http://doi.org/10.1016/j.ijpsycho.2005.10.002

Morgan, C. A., Wang, S., Mason, J., Southwick, S. M., Fox, P., Hazlett, G., … Greenfield, G. (2000). Hormone profiles in humans experiencing military survival training. *Biological Psychiatry*, *47*(10), 891–901. http://doi.org/10.1016/S0006-

3223(99)00307-8

Murphy, G. P., Mirand, E. A., Groenewald, J. H., & Kenny, G. M. (1970). Renoprival erythropoietin responsiveness in subhuman primates. *Journal of Surgical Oncology*, *2*(1), 9–16. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4999942

Neal, R. A., Prout, J., Costello, J., Massey, H. C., Tipton, M. J., Young, J. S., & Corbett, J. (2017). The effects of 10 days of separate heat and hypoxic exposure on heat acclimation and temperate exercise performance. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, (82), ajpregu.00103.2017. http://doi.org/10.1152/ajpregu.00103.2017

Nee, D. E., Wager, T. D., & Jondies, J. (2007). Interference resolution: Insights from a meta-analysis of neuroimaging tasks. *Cognitive, Affective, & Behavioural Neuroscience*, *7*(1), 1–17. http://doi.org/10.3758/CABN.7.1.1

Ochi, G., Kanazawa, Y., Hyodo, K., Suwabe, K., Shimizu, T., Fukuie, T., … Soya, H. (2018). Hypoxia-induced lowered executive function depends on arterial oxygen desaturation. *Journal of Physiological Sciences*, (0123456789), 1–7. http://doi.org/10.1007/s12576-018-0603-y

Ogoh, S., Tsukamoto, H., Hirasawa, A., Hasegawa, H., Hirose, N., & Hashimoto, T. (2014). The effect of changes in cerebral blood flow on cognitive function during exercise. *Physiological Reports*, *2*(9), e12163. http://doi.org/10.14814/phy2.12163

Owen, A. M., McMillan, K. M., Laird, A. R., & Bullmore, E. (2005). N-back working memory paradigm: A meta-analysis of normative functional neuroimaging

studies. *Human Brain Mapping*, *25*(1), 46–59. http://doi.org/10.1002/hbm.20131

Panjwani, U., Thakur, L., Anand, J. P., Malhotra, A. S., & Banerjee, P. K. (2006). Effect of simulated ascent to 3500 meter on neuro-endocrine functions. *Indian Journal of Physiology and Pharmacology*, *50*(3), 250–256.

Petrassi, F. A., Hodkinson, P. D., Walters, P. L., & Gaydos, S. J. (2012). Hypoxic hypoxia at moderate altitudes: Review of the state of the science. *Aviation Space and Environmental Medicine*, *83*(10), 975–984. http://doi.org/10.3357/ASEM.3315.2012

Pinti, P., Tachtsidis, I., Hamilton, A., Hirsch, J., Aichelburg, C., Gilbert, S., & Burgess, P. W. (2018). The present and future use of functional near-infrared spectroscopy (fNIRS) for cognitive neuroscience. *Annals of the New York Academy of Sciences*, 1–25. http://doi.org/10.1111/nyas.13948

Raichle, M. E., & Gusnard, D. A. (2002). Appraising the brain's energy budget. *Proceedings of the National Academy of Sciences*, *99*(16), 10237–10239. http://doi.org/10.1073/pnas.172399499

Roach, R. C., Hackett, P. H., Oelz, O., Bärtsch, P., Luks, A. M., MacInnis, M. J., … Zafren, K. (2018). The 2018 Lake Louise Acute Mountain Sickness Score. *High Altitude Medicine & Biology*, *19*(1), 4–6. http://doi.org/10.1089/ham.2017.0164

Robison, L. S., Gannon, O. J., Salinero, A. E., & Zuloaga, K. L. (2019). Contributions of sex to cerebrovascular function and pathology. *Brain Research*, *1710*(September 2018), 43–60. http://doi.org/10.1016/j.brainres.2018.12.030

Rostrup, M. (1998). Catecholamines, hypoxia and high altitude. *Acta Physiologica Scandinavica*, *162*(3), 389–399. http://doi.org/10.1046/j.1365-

201X.1998.00335.x

Schoofs, D., Preuß, D., & Wolf, O. T. (2008). Psychosocial stress induces working memory impairments in an n-back paradigm. *Psychoneuroendocrinology*, *33*(5), 643–653. http://doi.org/10.1016/j.psyneuen.2008.02.004

Subudhi, A. W., Miramon, B. R., Granger, M. E., & Roach, R. C. (2009). Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. *Journal of Applied Physiology*, *106*(4), 1153–1158. http://doi.org/10.1152/japplphysiol.91475.2008

Sudo, M., Komiyama, T., Aoyagi, R., Nagamatsu, T., Higaki, Y., & Ando, S. (2017). Executive function after exhaustive exercise. *European Journal of Applied Physiology*, *117*(10), 2029–2038. http://doi.org/10.1007/s00421-017-3692-z

Takano, N. (1984). Changes of ventilation and ventilatory response to hypoxia during the menstrual cycle. *Pflugers Archiv : European Journal of Physiology*, *402*(3), 312–316. http://doi.org/10.1007/BF00585515

Taylor, L., Watkins, S. L., Marshall, H., Dascombe, B. J., & Foster, J. (2016). The impact of different environmental conditions on cognitive function: A focused review. *Frontiers in Physiology*, *6*(JAN), 1–12. http://doi.org/10.3389/fphys.2015.00372

Tipton, M. (2012). A case for combined environmental stressor studies. *Extreme Physiology & Medicine*, *1*(1), 7. http://doi.org/10.1186/2046-7648-1-7

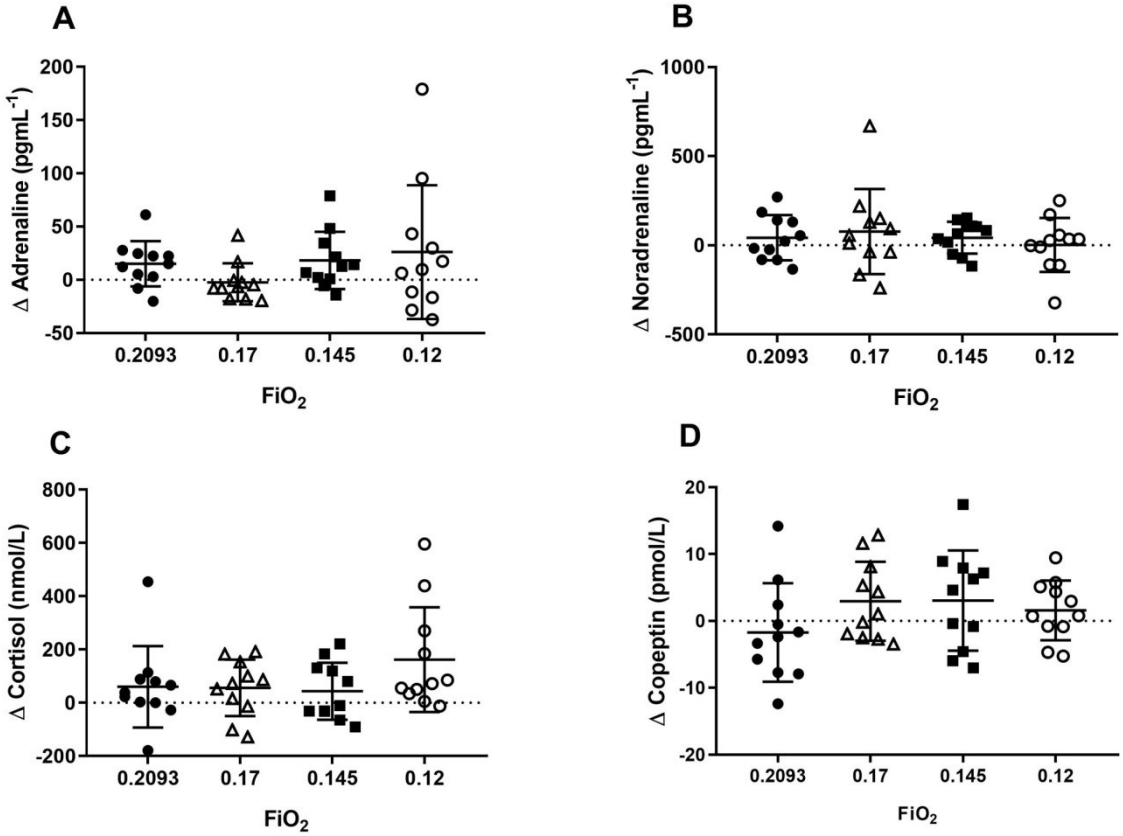
Tipton, M. J. (2016). Environmental extremes: origins, consequences and amelioration in humans. *Experimental Physiology*, *101*(1), 1–14. http://doi.org/10.1113/EP085362

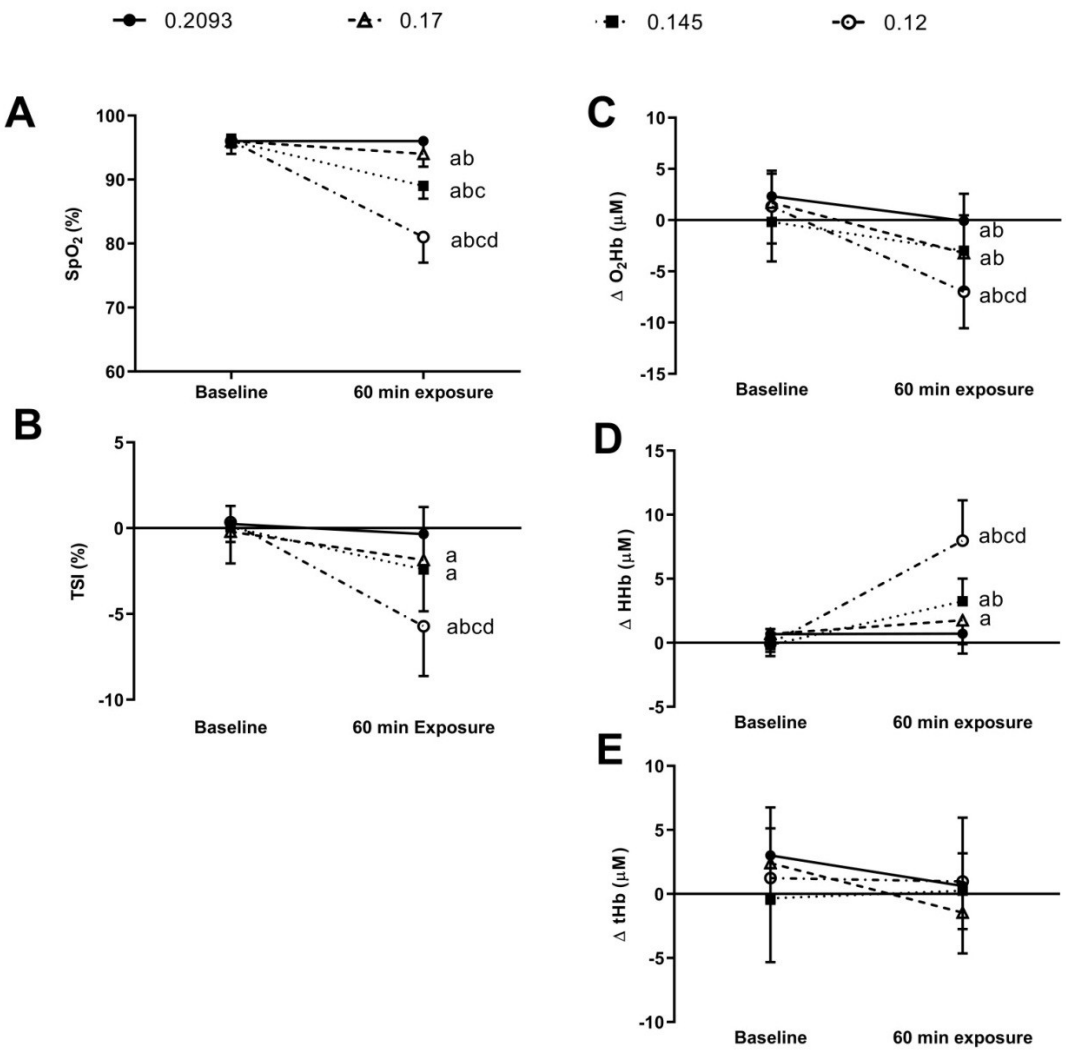
Virués-Ortega, J., Buela-Casal, G., Garrido, E., & Alcázar, B. (2004). Neuropsychological functioning associated with high-altitude exposure. *Neuropsychology Review*, *14*(4), 197–224. http://doi.org/10.1007/s11065-004-8159-4

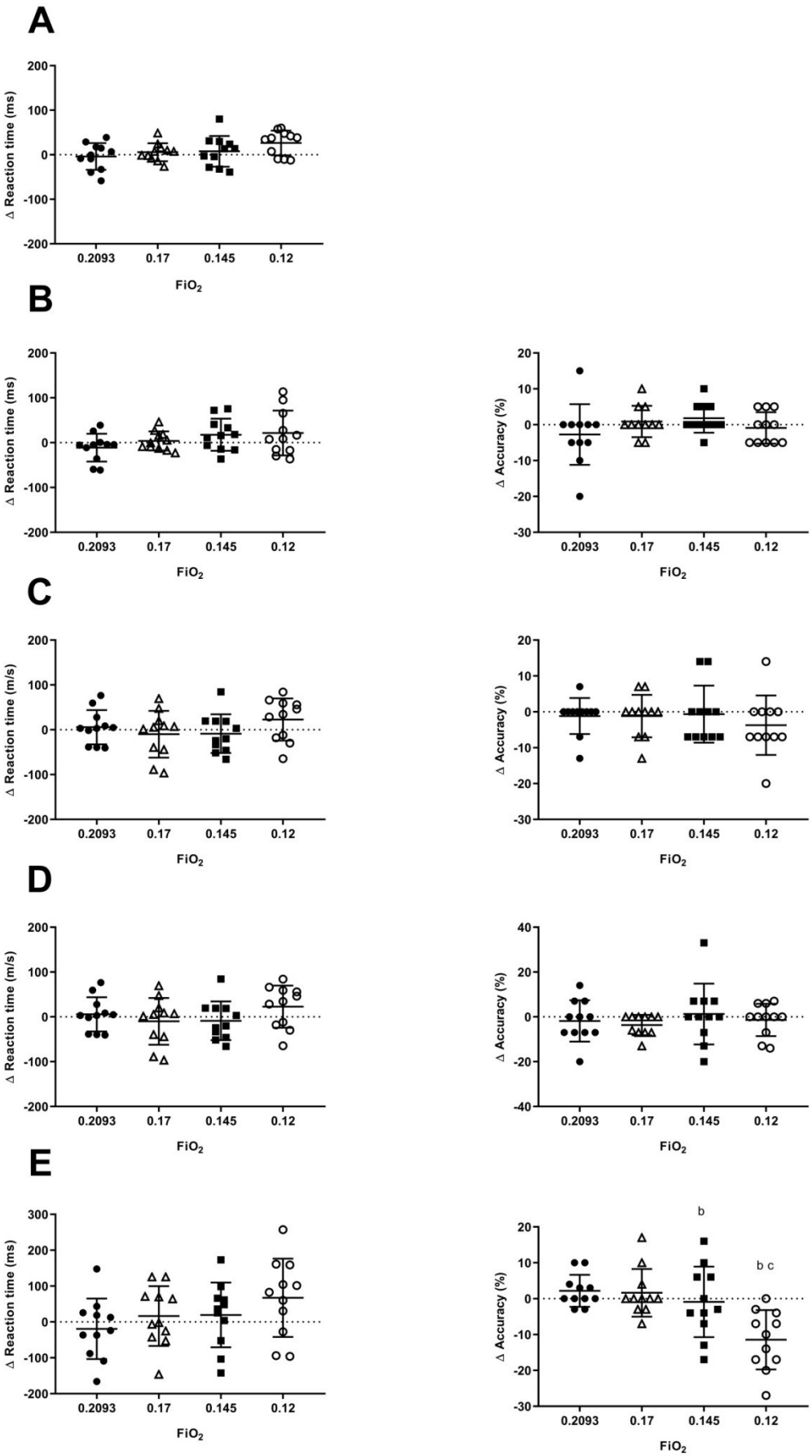
Willie, C. K., Macleod, D. B., Shaw, A. D., Smith, K. J., Tzeng, Y. C., Eves, N. D., … Ainslie, P. N. (2012). Regional brain blood flow in man during acute changes in arterial blood gases. *Journal of Physiology*, *590*(14), 3261–3275. http://doi.org/10.1113/jphysiol.2012.228551

Wilson, R. C., & Jones, P. W. (1989). A comparison of the visual analogue scale and modified Borg scale for the measurement of dyspnoea during exercise. *Clinical Science*, *76*(3), 277–282. http://doi.org/10.1042/cs0760277

Yan, X. (2014). Cognitive Impairments at High Altitudes and Adaptation. *High Altitude Medicine & Biology*, *15*(2), 141–145. http://doi.org/10.1089/ham.2014.1009







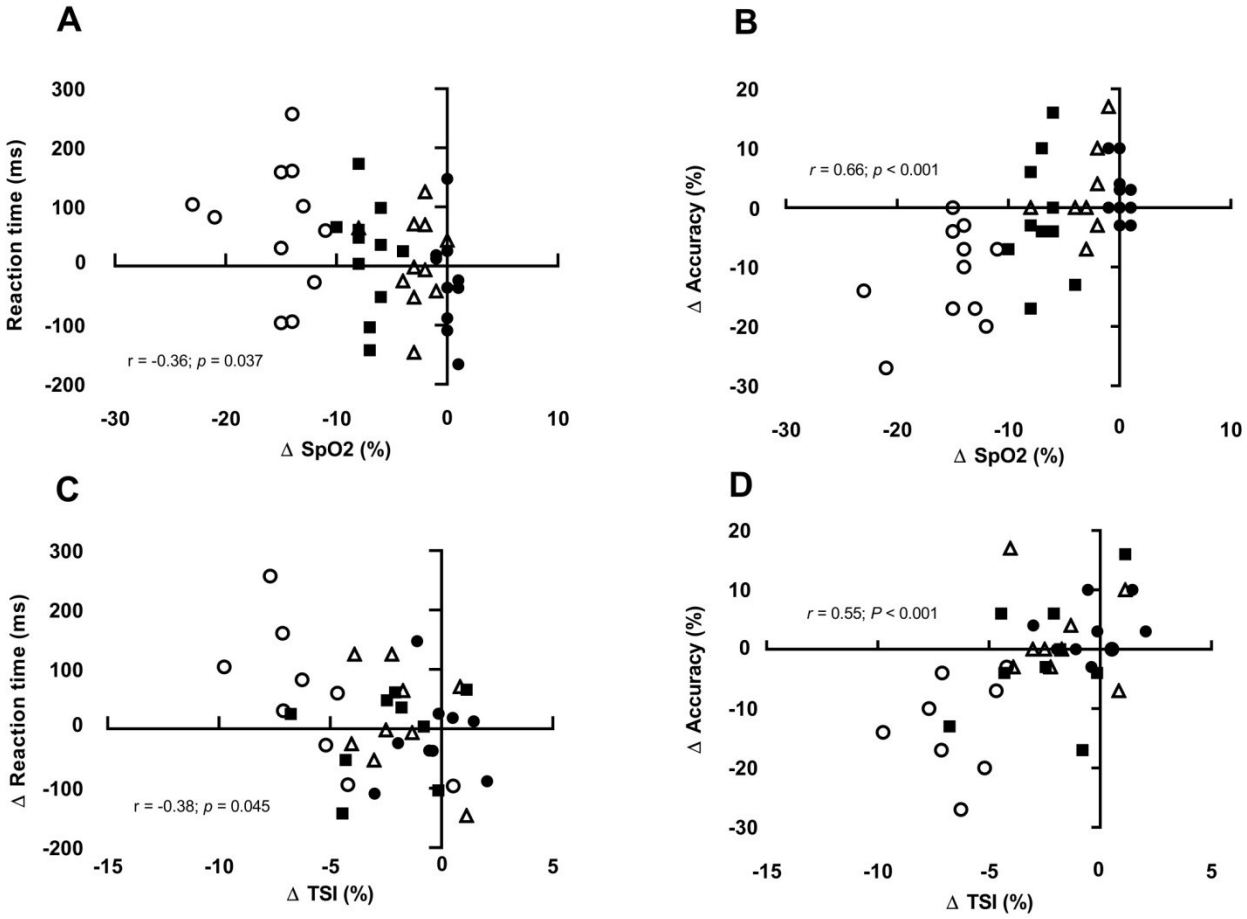


Table 1. Reaction time and accuracy for the cognitive tests at baseline and following 60 minutes of exposure (n=11).

	FiO_2								*p* value		
	0.2093		0.17		0.145		0.12		FiO_2	Time	Interaction
	Baseline	Exposure	Baseline	Exposure	Baseline	Exposure	Baseline	Exposure			
Simple reaction time											
Reaction time (ms)	257 [26]	253 [14]	244 [13]	250 [19]	261 [28]	269 [11]	241 [15]	282 [28]	**0.028**	**0.024**	0.142
Choice reaction time											
Reaction time (ms)	381 [48]	370 [50]	353 [33]	356 [36]	352 [37]	370 [50]	358 [43]	380 [52]	0.153	0.700	0.159
Accuracy (%)	98 [5]	95 [7]	96 [6]	97 [3]	95 [4]	97 [3]	97 [3]	96 [3]	0.940	0.837	0.172
Eriksen Flanker											
Congruent reaction time (ms)	532 [91]	517 [88]	527 [75]	524 [76]	523 [77]	510 [87]	515 [78]	517 [59]	0.765	0.406	0.831
Congruent accuracy (%)	99 [2]	98 [5]	96 [9]	96 [6]	96 [7]	96 [4]	99 [3]	95 [6]	0.253	0.256	0.622
Incongruent reaction time (ms)	544 [74]	550 [68]	568 [83]	558 [101]	552 [85]	543 [84]	534 [61]	556 [64]	0.504	0.795	0.184
Incongruent accuracy (%)	94 [9]	92 [12]	96 [5]	93 [6]	93 [11]	93 [9]	94 [7]	92 [6]	0.875	0.330	0.671
N-back											
Reaction time (ms)	598 [125]	578 [95]	614 [146]	631 [106]	616 [109]	636 [109]	613 [129]	680 [131]	0.545	0.164	0.204
Accuracy (%)	90 [6]	92 [3]	89 [8]	91 [6]	86 [9]	**85 [10]**[b]	**93 [5]**[d]	**82 [9]**[abc]	0.167	0.152	**< 0.001**

Values are mean [SD]. [a] significantly different ($p < 0.05$) to the baseline value in the same environment. [b] significantly different ($p < 0.05$) to same time point in FiO_2 0.2093. [c] significantly different ($p < 0.05$) to same time point in FiO_2 0.17. [d] significantly different ($p < 0.05$) to same time point in FiO_2 0.145.

Table 2. Cardiorespiratory responses and subjective outcome measured during the cognitive tasks at baseline and after 60 minutes of exposure (n = 11).

	FiO_2								*p* value		
	0.2093		0.17		0.145		0.12		FiO_2	Time	Interaction
	Baseline	Exposure	Baseline	Exposure	Baseline	Exposure	Baseline	Exposure			
HR ($b.min^{-1}$)	70 [9]	**65 [9]**[a]	69 [12]	69 [12]	72 [10]	**74 [10]**[bc]	71 [11]	**76 [13]**[abc]	**0.049**	0.617	**< 0.001**
$\dot{V}_E$ ($L.min^{-1}$)	14.2 [1.2]	12.3 [2.0]	13.5 [2.4]	12.5 [2.6]	14.1 [1.8]	13.8 [2.6]	13.4 [2.9]	13.4 [3.6]	0.693	**0.038**	0.121
V_T (L BTPS)	0.801 [0.139]	0.892 [0.423]	0.754 [0.132]	0.807 [0.276]	0.818 [0.173]	0.836 [0.122]	0.809 [0.156]	0.913 [0.247]	0.540	0.175	0.262
f_R (breaths. min^{-1})	18.0 [2.3]	15.5 [4.3]	18.1 [2.4]	16.4 [3.8]	17.5 [2.6]	16.8 [4.2]	16.9 [3.6]	15.2 [4.6]	0.202	**0.016**	0.583
$P_{ET}CO_2$ (mmHg)	38 [2]	37 [2]	38 [2]	**37 [2]**[a]	38 [2]	**37 [2]**[a]	38 [2]	**35 [4]**[abc]	0.176	**0.001**	**0.007**
$P_{ET}O_2$ (mmHg)	109 [2]	108 [2]	104 [5]	**78 [3]**[ab]	103 [6]	**61 [4]**[abc]	101 [7]	**48 [2]**[abcd]	**< 0.001**	**< 0.001**	**< 0.001**
AMS	0 [1]	1 [2]	0 [1]	1 [2]	0 [0]	0 [2]	1 [1]	2 [2]	0.267	**0.004**	0.694
Dyspnoea	0 [0]	0 [1]	0 [0]	0 [1]	0 [0]	0 [1]	0 [0]	1 [2]	**0.041**	0.078	0.099
Mood Disturbance	34 [6]	39 [11]	33 [13]	44 [23]	36 [10]	43 [24]	37 [13]	42 [26]	0.118	0.002	0.186

Cardiorespiratory values are are mean [SD]. HR, heart rate; $\dot{V}_E$, minute ventilation; f_R, respiratory frequency; V_T, tidal volume; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; $P_{ET}O_2$, end-tidal partial pressure of carbon dioxide. Subjective outcome measures are median [IQR]. AMS; acute mountain sickness. [a] significantly different ($p < 0.05$) to the baseline value in the same environment. [b] significantly different ($p < 0.05$) to same time point in FiO_2 0.2093. [c] significantly different ($p < 0.05$) to same time point in FiO_2 0.17. [d] significantly different ($p < 0.05$) to same time point in FiO_2 0.145.