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Effects of Fluctuations in Oxygen Partial Pressure from Normoxia to Moderate Hyperoxia

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STATEMENTS

Disclaimer

The views expressed in this technical report are those of the author and donot necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

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Human Research/Institutional Review Board (IRB) statement

The study protocol was reviewed by the Naval Medical Research Unit Dayton Institutional Review Board in compliance with all applicable federal regulations governing the protection of human subjects.

Animal Care and Use statement

N/A

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ABSTRACT

Pilots in modern tactical aircraft breathe oxygen-enriched gas supplied by onboard oxygen generating systems (OBOGS). These may provide their maximum possible oxygen fraction at all times (nominally 94%), or, like that in the F-35, control the oxygen fraction at lower concentrations on a schedule based on altitude. The altitude-based schedules have a wide band of acceptable oxygen concentrations, and OBOGS that follow them may supply gas concentrations that fluctuate within the acceptable band. This IRB-approved study addressed a concern that unsteady oxygen partial pressure might be related to physiological episodes (PEs). Oxygen concentrations of 21% (air) and 35% were supplied on the ground to approximate operational conditions in which the inhaled partial pressures fluctuate by 15% from normoxia to slight hyperoxia at a cabin altitude of 8,000 feet. Twenty-five healthy adults provided written informed consent and completed four sessions on separate days that were identical except for breathing gas. At each of two visits, a steady gas concentration of either 21% or 35% oxygen was delivered. For the other two visits, participants breathed a steady concentration of one or the other gas followed by a period when the concentration of oxygen switched every 30 seconds between these concentrations. Middle cerebral artery bloodflow velocity (transcranial Doppler;TCD), frontal hemoglobin oxygenation (near infrared spectroscopy; NIRS), vigilance, short-term memory, and parameters of breathing were measured during the above periods and during initial baseline and ending recovery periods breathing air at each visit.

The principal findings of this study were that neither steady breathing of 35% O_2 for 50 minutes, nor 30-second fluctuations in inhaled oxygen percentage between 21% and 35%, caused significant changes in middle cerebral artery flow velocity as measured using TCD. Return to 21% O_2 was equally uneventful. Similarly, there was no decrease in oxygenation in the frontal cortex as measured by NIRS. There was a consistent decrease in frontal cortex HHb when participants breathed steady 35% or fluctuated between 21% and 35%, but this was anticipated. Vigilance and short-term memory performance were not impaired or facilitated, and no consistent set of symptoms was reported. To extrapolate these findings to equivalent oxygen concentrations in tactical aircraft, fluctuations with the gas changing every 30 seconds between 30% and 45% O_2 at a cabin altitude of 8,000 ft MSL would not appear to affect oxygen delivery to the brain.

INTRODUCTION

Pilots in Navy high-performance jets are supplied with nominally 94% to 100% oxygen through their life support systems. However, Air Force pilots and the Navy pilots who fly the F-35 are supplied with lower oxygen fractions when the altitude permits. Their life support systems follow an oxygen schedule designed to prevent hypoxia while including nitrogen in the breathing gas to prevent some of the problems caused by a very high oxygen fraction in the breathing gas, for example, symptoms suggestive of absorption atelectasis.

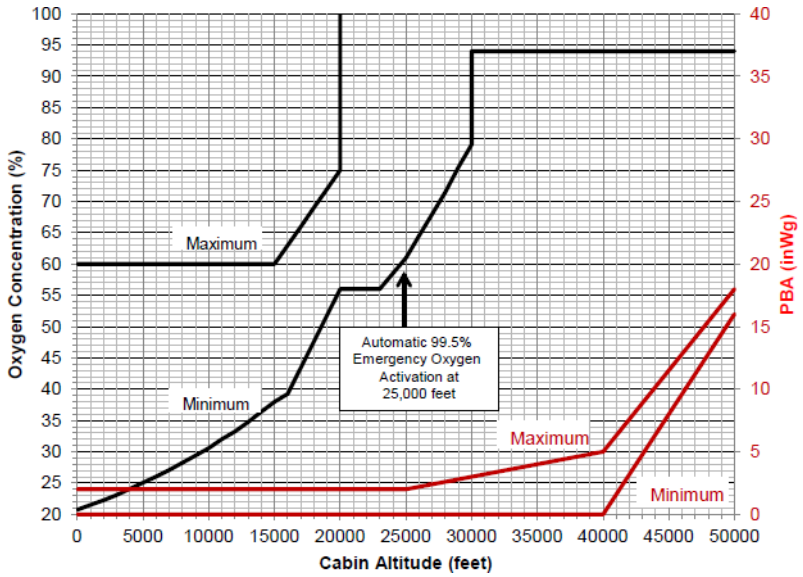

Figure 1. The oxygen schedule for an aircraft that flies to 50,000 ft with a 5 psi cabin differential pressure (1). Oxygen concentrations that fall between the black lines are acceptable. The red lines similarly indicate the acceptable ranges of regulator output pressure.

Oxygen schedules, like the example shown from MIL STD 3050 (Figure 1), have bands of acceptable oxygen fractions, hence of oxygen partial pressures, as a function of cabin altitude. For Air Force planes at cabin altitudes not greater than 15,000 ft mean sea level (MSL) the maximum is 60% oxygen, while for the F-35 at cabin altitude not exceeding 11,000 ft MSL, the maximum is 65% oxygen. The minimum is set to maintain an inhaled oxygen partial pressure slightly higher than that from breathing air at sea level.

Many OBOGS always deliver the maximum oxygen concentration possible for the conditions. Although their oxygen output is not always the nominal 94% and sudden drops may occur, those drops are neither regular nor cyclic. However, some OBOGS

are designed with two modes, one for maximum oxygen fraction, and an additional control mode that satisfies the oxygen schedule for their aircraft. In controlled mode, regular, quasi-sinusoidal oscillations often appear, with periods from 30 to 60 seconds and with peak-to-peak magnitude about 15% oxygen. (When the system is not stressed, these correspond to cycling times of the oxygen-concentrator beds.) Barring any contradictory information, any oxygen fraction in the acceptable range of the oxygen schedule, steady or rippling around an average value, could be assumed to be satisfactory. However, effects of oscillations had not been measured, and the military aviation community asked whether they might be harmful (2).

Long-term hyperoxia causes vasoconstriction. Some studies have suggested that responses to normoxia after hyperoxia might mimic responses to hypoxia following normoxia (3). Thus, there was a concern that oxygen fluctuations could lead to relative hypoxia in the brain despite the apparently-adequate inhaled oxygen partial pressures (PO_2) (4). Accordingly, NAMRU-D conducted experiments to test the effects of regular fluctuations in PO_2 with a period of 60 seconds, that is, with 30 seconds per gas composition.

Oxygen partial pressure, not oxygen fraction, drives physiological responses; equivalent in-flight partial pressures were matched on the ground. Although most in-flight oxygen fluctuations remain within the oxygen schedule where normoxic gas generates a warming and thus range from slightly- to moderately hyperoxic, this study used normoxic to moderately hyperoxic fluctuations to increase the chance of physiologic effect with the observed 15% amplitude of oxygen variation. The cabin altitude of 8,000 ft above MSL was targeted; many tactical aircraft maintain that pressure (cabin altitude) for most of their flying ranges. Normoxia at 8,000 ft MSL is achieved with 30% O_2. Thus, fluctuations from normoxia to an oxygen fraction 15% higher became 30% to 45% O_2 at altitude, that is, between air (21% O_2) and 35% O_2 in the laboratory.

The principal hypothesis was that a person who had equilibrated to an elevated oxygen content and then breathed gas that fluctuated to lower oxygen concentrations might experience insufficient oxygen delivery, while a person equilibrated to air who experienced the same fluctuations might have normal oxygen delivery during both the normoxic and the hyperoxic phases of the fluctuation. Tests were conducted with 21% oxygen and then fluctuations, with 35% oxygen and then fluctuations, with 35% oxygen without fluctuations, and, as a time control, with 21% oxygen without fluctuations. Middle cerebral artery velocity (MCAv) was selected as a surrogate measure of brain blood flow. MCAv can be measured using transcranial Doppler ultrasound (TCD) through the temporal window (between the ear and the eyebrow). The artery lies at a depth of 30 to 65 mm, depending on the individual, and flows towards the side of the head. Near infrared spectroscopy (NIRS) on the forehead was chosen to measure oxygenation of the frontal areas of the brain. Additional variables measured included brachial blood pressure, heart rate, end tidal PO_2 and PCO_2 (partial pressure of carbon dioxide) as indicators of alveolar gas, and respiratory variables (e.g., frequency, tidal volume, and minute ventilation). Cognitive effects of fluctuations in gas composition also were assessed.

METHODS

Flyers and online announcements were used to recruit healthy adults ages 18-45 years. Participants who completed the study received $600. The study protocol was approved by the Naval Medical Research Unit – Dayton's Institutional Review Board in compliance with all applicable federal regulations governing the protection of human participants. All participants self-reported normal vision or vision corrected-to-normal with contact lenses, no history of psychological, neurological, or medical diagnosis, no use of tobacco in the past 6 months, and no excessive alcohol use.

Although 47 volunteers consented to participate, eight took part in an early pilot series from which data were not used, four were lost to the study after the COVID-19 shut-down, and in nine, TCD signals could not be found in a reasonable length of time. Twenty-six people, 16 men and 9 women, completed all 4 data collection sessions. However, TCD was lost during an experiment for one of the men. The 25 participants whose data are reported here ranged in age from 19 to 42 (median 29) years, in height from 73 to 196 (median 176) cm, and in weight from 60 to 105 (median 84) kg.

Seated participants were exposed to four conditions, one per visit, in a counter-balanced, within-subjects design. Visits were separated by at least three days. The structure of each visit is shown in Figure 2: 1) Baseline: five minutes breathing 21% O_2; 2) Phase 1/ conditioning: 20 minutes breathing 21% or 35% O_2; 3) Phase 2: 30 minutes during which the gas switched every 30 seconds ("fluctuations", [F]) or remained constant ("steady", [S]) at the concentration of the conditioning phase, and 4) Phase 3: 30 minutes breathing 21% O_2. Gas conditions will be denoted 21%O_2S, 21%O_2F, 35%O_2S, and 35%O_2F. Late in each gas exposure when participants were presumed to be in a hemodynamic steady state they performed a visual fixation task.

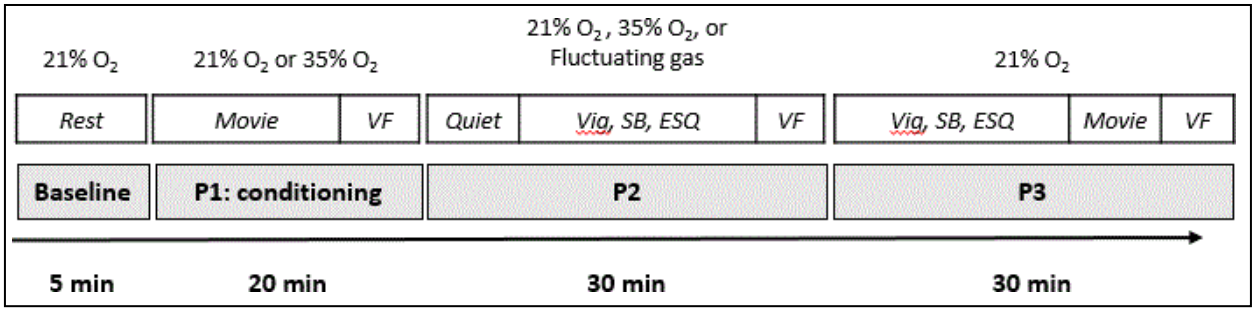

Figure 2: Timeline for data collection sessions. Shaded blocks=phases. Unshaded blocks=participant activities during each phase: During VF (visual fixation), participants were asked to sit still and relaxed while focusing on a dark background with a light plus sign in the center. "Quiet" indicates five minutes with no tasks or movie; "Vig", a vigilance task; "SB" a Sternberg letter sequence test, and "ESQ", an Environmental Symptoms Questionnaire.

Instrumentation

Middle cerebral artery velocity was measured using transcranial Doppler M-mode ultrasound (TCD) (MultiDop T, Compumedics, Singen, Germany). Bilateral 2 MHz pulse wave probes incorporated into the DWL Diamon headband were placed over the trans-temporal windows. In our population, TCD signals were found at depths between 36 and 68 mm. The median depth was 52 mm.

Near infrared spectroscopy (NIRS) measured relative changes in oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (HHb) in the frontal cortex. Data were recorded via bilateral Artinis PortaLite NIRS (Artinis Medical Systems, Elst, The Netherlands). Sensors were placed on the forehead at sites Fp1 and Fp2 according to he International 10-20 System for electroencephalogram placement (5).

Participants wore a fitted silicone oronasal mask (Series 7450, Hans Rudolph, Shawnee, KS) connected to a one-way non-rebreathing valve assembly (Model 2700, Hans Rudolph). The inlet to the valve assembly was attached to the outlet port of a three-way balloon valve block (2540 series, Hans Rudolph). Breathing gases were supplied at atmospheric pressure to two inlet ports of the balloon valve block via large-bore (35 mm id) respiratory tubing (VacuMed, Ventura CA) from two gas reservoirs (60 L gas bag, Hans Rudolph). The third port of the valve block opened to room air. The gas reservoirs were filled manually from gas cylinders of compressed air (21% O_2) or 35% O_2 in N_2. The balloon valves were manipulated using a control unit (2530 series, Hans Rudolph) activated with a purpose-written LabVIEW (National Instruments, Austin, TX) timer program. The mask assembly was held in place with Hans Rudolph V2 adjustable headgear placed over the TCD headband, and the valve blocks were supported at a height comfortable for the participant. An eye-level LCD screen showed computer tasks and, during down-time, a movie. Participants used a mouse or numeric key pad with their right hands for the computer tasks.

Oxygen and carbon dioxide fractions in the mask were measured using a fast-response gas analyzer (GA-200, iWorx, Dover NH) using no filters and with the gas sampling pump set to 400 mL/min. The sample line was inserted through a port between the mask and the valve assembly until the end was approximately centered in the circular ring in the stream of gas flow to and from the mask.

Transcutaneous CO_2 and O_2 partial pressures were measured using a TCM4/ CombiM84 (Radiometer, Copenhagen, DK) with its electrode placed on the left upper chest. Finapres Nova (Finapres Medical Systems, Enschede, The Netherlands) finger cuffs on the left third and fourth fingers and arm cuff on the left upper arm measured continuous beat-to-beat finger-, and intermittent brachial artery blood pressure, respectively, following an initial 5-minute stabilization period. However, the only blood pressure data used were the intermittent brachial cuff pressures, because brachial blood pressure measurements were manually initiated during some of the periods chosen for data analysis and the finger pressures were not calibrated to brachial pressure after all finger changes. (See DISCUSSION.)

A pressure transducer (range ± 1 psi, SSC series, Honeywell SIT, Fort Mill SC) attached to a port on the mask's connecting ring measured the pressure in the mask. Inspiratory flow was measured in the gas supply tubes near the gas reservoir outlets using screen pneumotachometers (Hans Rudolph model 4719, linear to 100 L/min, signal pressure 1 cm H_2O at 100 L/min,) and associated pressure transducers (range ± 1 inH_2O, SSC series, Honeywell SIT).

Continuous physiological data, except NIRS, were sampled at 500 Hz, displayed, and stored using a PowerLab/LabChart data acquisition suite (ADInstruments, Colorado Springs, CO) and a computer. NIRS data were sampled at 10 Hz and collected on a second computer. Computer tasks were presented and the data were stored on a third computer using a program written for the purpose in MATLAB with Psychtoolbox extensions (Mathworks, Natick, MA).

Cognitive tasks and symptoms questionnaire

Vigilance Task

To assess vigilance, participants were presented with a challenging discrimination task based on the Abbreviated Vigilance Task (6). On a computer display, a single central character was shown in low contrast in the center of a computer screen with a noisy background. The character presented was a letter "D", a backwards "D", or an "O". Participants were instructed to respond with a keypress if they saw an "O" (i.e., a critical trial), but to withhold their response if they saw a "D" or backwards "D" (i.e., a neutral trial). The stimuli were presented on the screen for 70 ms and then masked for 1 second. The mask was then followed by a 1-second inter-trial interval (ITI). Participants could respond at any point during the post-stimulus mask or the ITI. A total of 300 trials were presented; 20% were critical trials and 80% were neutral trials. Accuracy for both trial types was analyzed along with critical trial response time (RT). The task took 10 minutes to complete. Participants performed the vigilance task twice, once during each of Phases 2 and 3.

Sternberg Task

To assess short-term memory performance, a Sternberg memory task (7, 8) was employed. At each administration, participants were presented with a novel string of 7 letters (e.g., FVPGCQY) for 15 seconds. Following this initial memorization phase, participants were presented with a single letter and asked to judge whether or not the letter was part of the original list. Half of trials presented a letter that was part of the original list and the other half presented a letter that was not. Participants completed 60 trials per administration. Both accuracy and RT were measured. The task took approximately 4 minutes to complete. Participants completed the Sternberg task twice, once during each of Phases 2 and 3 following completion of the vigilance task.

Environmental Symptoms Questionnaire

The Environmental Symptoms Questionnaire (ESQ) is a 68-item assessment of potential symptoms (e.g., I feel lightheaded, I feel alert) developed and validated to judge symptoms of high-altitude sickness (9). The participants rated current symptoms on a 0 to 5 scale (0 = not at all, 5 = extreme). The ESQ has nine separate sub-scales or symptom clusters: cerebral, respiratory, ears/nose/throat (ENT), cold, distress, alertness exertion, muscle, and fatigue. Participants were administered the ESQ three times, just before the start of the Baseline phase, then during Phases 2 and 3 following vigilance and Sternberg tasks.

Periods of data analysis

Data for cardiovascular (TCD) analyses were extracted from LabChart files by identifying cardiac cycles from the stronger of the left and right MCAv signals. Periods were selected to start and end on a minimum (end diastole). Other LabChart signals, for example, end-tidal oxygen, also were assessed during the period selected from the MCAv, with the software configured as appropriate to average cyclic maxima, minima, or means across the periods chosen, or to average a signal over the entire period.

TCD and NIRS data were examined across four separate periods: 1) visual fixation periods, for steady state relative to the gas conditions and with minimal mental activation; 2) the vigilance tasks; 3) the Sternberg tasks, and 4) the first 5 minutes of Phase 2 for the start of the fluctuations and their time-matched steady gas periods. TCD was also studied at the start of Phase 3, the step change back to (or continuation of) air breathing.

During each of the four, 5-minute visual fixation periods, approximately one minute of data was selected, between minutes 3.5 and 4.5 of each (see Figure 2). For the vigilance tasks, TCD data were selected in two 5-minute periods that were averaged for the statistical analysis to match the NIRS analysis across the entire 10-minute task. For the Sternberg tasks, both TCD and NIRS signals were selected to span the entire period of task performance. The first five minutes of Phase 2 was split into epochs, ten for the TCD data, and five for the more-slowly sampled NIRS data. TCD data were selected for as large an integral number of cardiac cycles as possible for each epoch, for 21%O_2F and 35%O_2F from last 20 seconds of each gas step, and for 21%O_2S and 35%O_2S, from the full 30-second segment. NIRS data were selected based on time from the start of the phase. TCD data for the first five minutes of Phase 3 also were examined in 30-second epochs. This period corresponded to the first half of the second vigilance task.

Data reduction and analysis

Gas switching

The rapidity with which inspired gas switched was assessed by examining a few individual fluctuations on a breath-by-breath basis. Data were extracted from LabChart files between peaks of expired CO_2; inspired gas was measured coincident with the

minima, and expired gas coincident with the maxima, of CO_2.

Time for a new steady state after a step change in gas was assessed at the start of Phase 3 using repeated measures analysis of variance (ANOVA), gas condition x epoch, with follow-up analysis by one-way repeated measures ANOVA by epoch within gas condition and contrasts to compare individual epochs with the steady-state value.

TCD

A single mean MCAv, the left or right, was selected from each individual data set for statistical comparisons. The primary criterion was the absence of noise, and the secondary one, signal amplitude at the end of data acquisition. Because the heart rate corresponding to the MCAv indicates the fidelity of the selection of beat-to-beat maxima and minima, noise was detected automatically by comparing the heart rates as computed from the two MCAv signals with that from the finger blood pressure. Data segments in which one of the heart rates differed from the average of the three by more than 10% were flagged for inspection, whereupon any MCAv signal with poorly-differentiated heart beats was removed from analysis. When both left and right signals were clean, the MCAv with larger amplitude during visual fixation at the end of Phase 3 was used. Pulsatility Index (PI) and vascular conductance index (CI; defined as MCAv/MAP) when MAP was available also were analyzed from the selected TCD signal.

MCAv and CI were expressed as the fraction of baseline value (f_MCAv, f_CI) for the gas condition (participant visit) to eliminate differences caused by the angle of insonification (see DISCUSSION). This reduced the effective number of phases from four to three. The PI, a ratio of two MCAv measurements, removes the effect of this angle in its calculation, and could be examined across all four phases, that is, including baseline.

Differences for the periods of visual fixation, vigilance and Sternberg testing were examined using two-way repeated-measures ANOVA. The analysis was of gas condition x phase, which for the periods of the vigilance and Sternberg tests, corresponded to gas condition x test order. Transient differences at the starts of Phases 2 and 3 were assessed using repeated measures ANOVA on epoch x gas condition. Individual differences were identified in post hoc testing using Tukey Honestly Significant Difference (HSD). A main effect of phase or test order with no interaction between phase and gas condition indicates changes with time independent of the gas supplied to the participant. A main effect of gas condition with no interaction would indicate a difference among days rather than a difference related to the gas breathed, because all gas conditions included an air-breathing baseline (not separately assessed for f_MCAv and f_CI) and an air-breathing Phase 3. Only a significant interaction of gas condition and phase would indicate a change relative to the gas inhaled.

NIRS

One participant was excluded from NIRS analysis due to missing data. Each analysis approach is described in more detail below.

The dependent variables of interest for NIRS, O_2Hb and HHb, are relative measures that were sampled at three separate depths from the right and left sensors. The analyses average across these three depths and across both sensors. Any significant condition x phase interactions were followed-up by planned one-way ANOVAs on condition for each phase. Significant main effects were then followed up with planned post-hoc comparisons, corrected for multiple comparisons with a Šidák correction.

RESULTS

Gas Mixing

The gas supplied to the mask switched between 21% and 35% O_2 in a single breath, while end-tidal (alveolar) composition changed more slowly (Figure 3). End tidal oxygen concentration for this person during the conditioning phases was 15% when 21% was inhaled, and 27% when 35% was inhaled. However, alveolar gas did not reach those steady-state levels within a 30-second period.

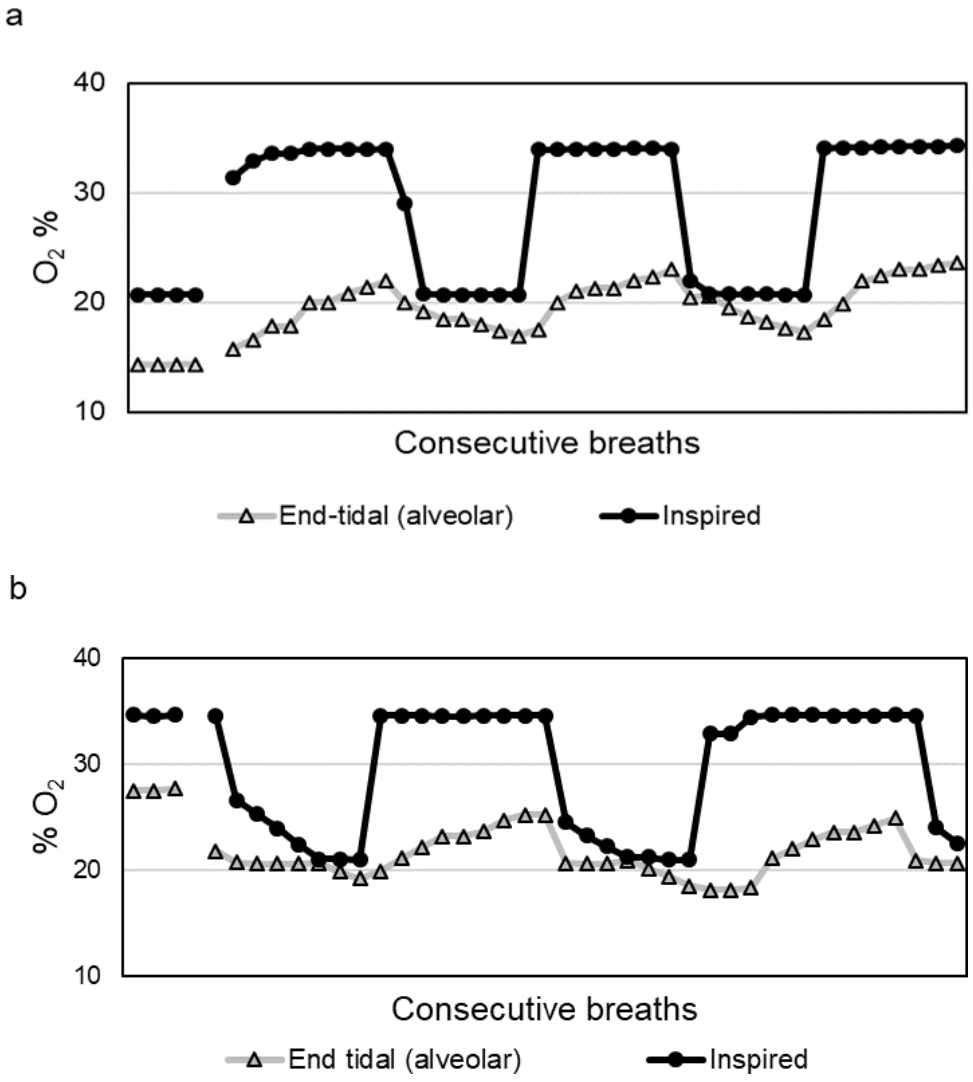

Figure 3. Breath-by-breath oxygen percentage as a function of time, inspired and end-tidal gas for one person. Each dot represents one breath. a) 21%O_2F, b) 35%O_2F. The first breaths shown (before the break in the lines) are from the end of Phase 1 when gas concentration was steady.

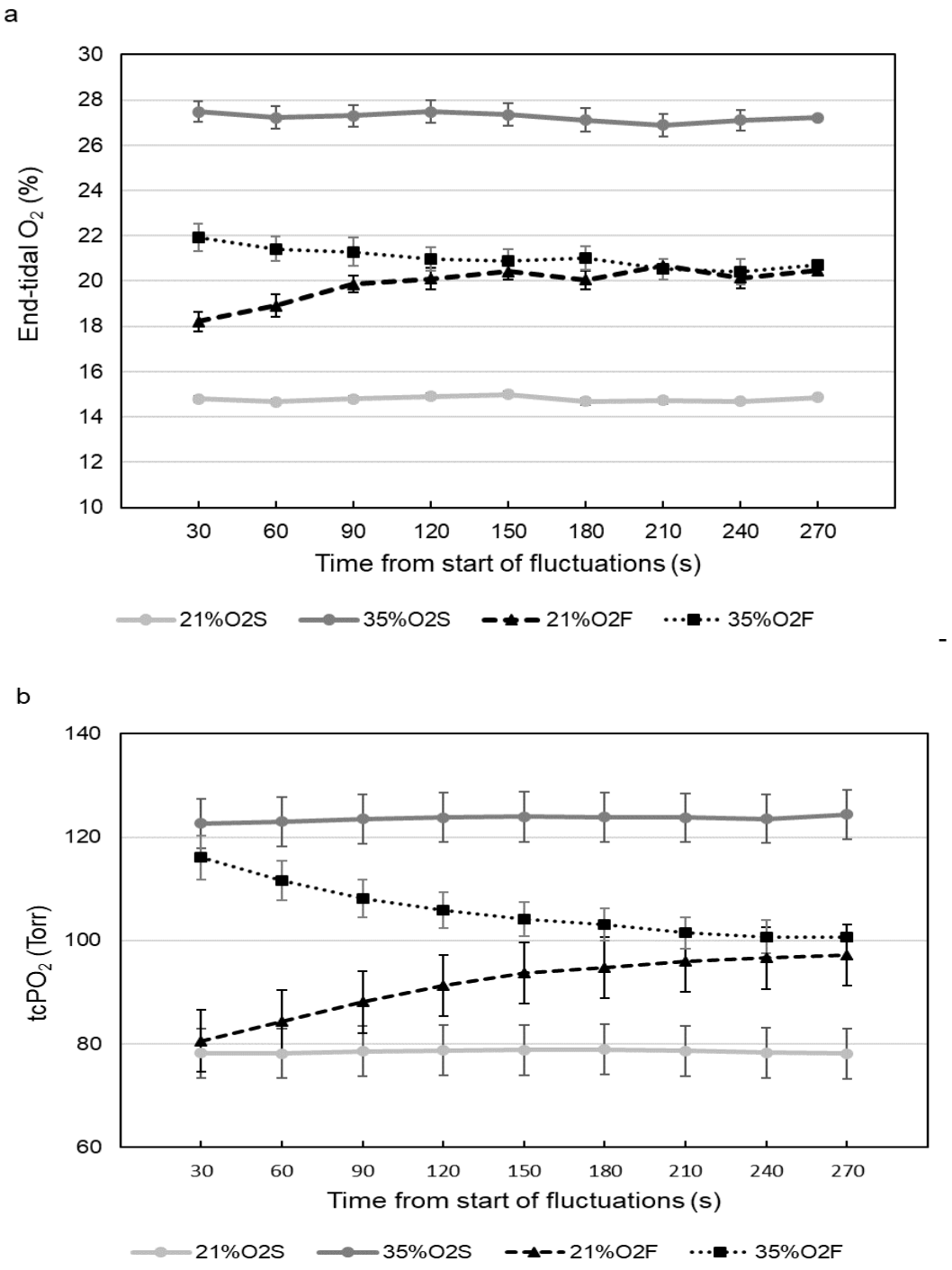

Figure 4. End-tidal oxygen fraction and $tcPO_2$ as functions of time for the first 4 minute of Phase 2, mean and standard error. Data are the 20 second (fluctuation) or 30 second (steady) averages obtained through selection basedon MCAv data.

The repeated step changes in inspired gas produced alveolar gas concentrations converged to values rippling around a value halfway between the two steady gas conditions (Figure 4a). The 21%O_2F and 35%O_2F end-tidal values became nearly equivalent (lines cross with each gas switch) after approximately three minutes with fluctuations. Because the two conditions are out of phase, no statistics are presented, as an analysis by epoch could mislead. Both end-tidal oxygen fraction and tcPO_2 for 21%O_2F and 35%O_2F converged to values halfway between those of 21%O_2S and 35%O_2S.

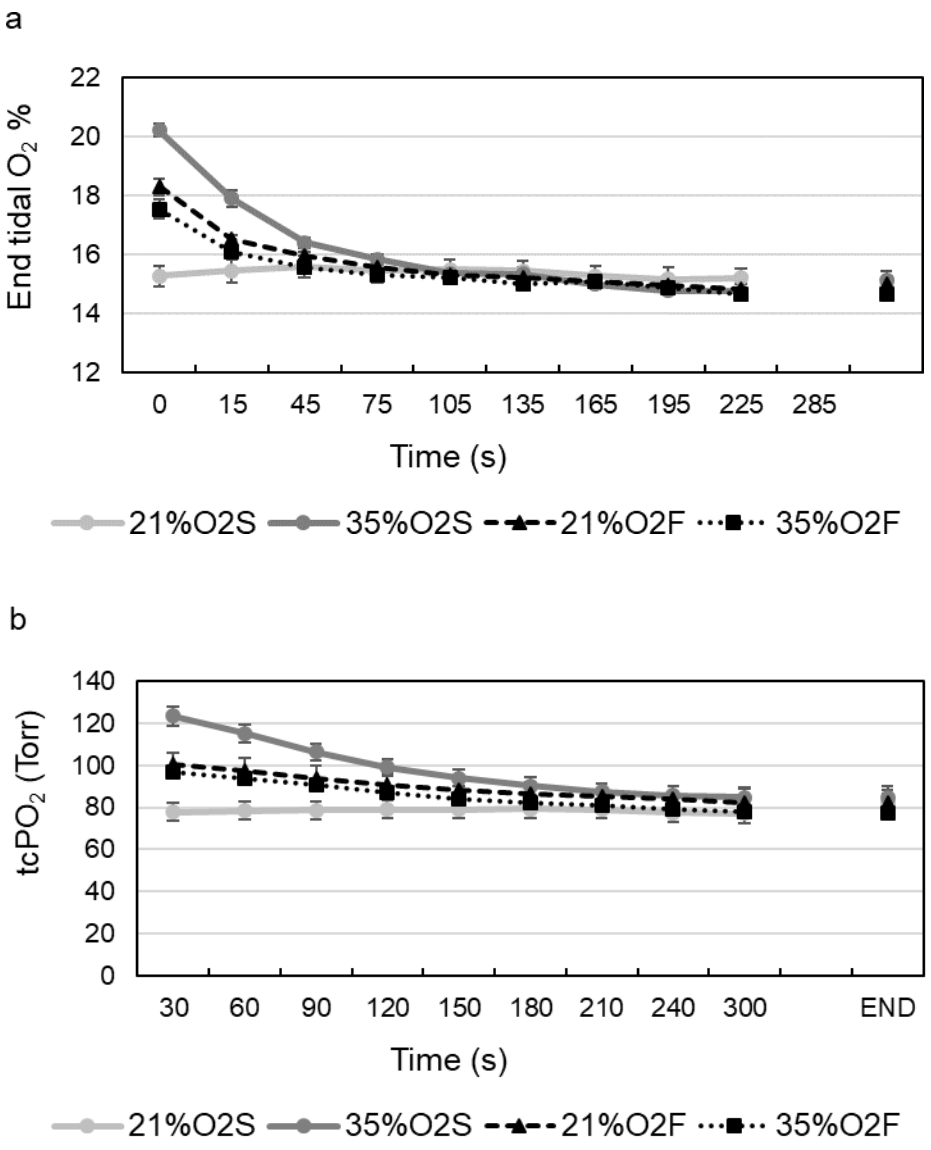

Figure 5. End-tidal oxygen fraction and tcPO_2 as functions of time in the first 4 minutes of Phase 3, mean and standard error. Data are 30 second averages obtained through selection based on MCAv data.

The start of Phase 3 represented a step change to 21% inspired oxygen for all conditions except 21%O_2S. Figure 5 shows resulting end-tidal and transcutaneous oxygen data.

The gas condition X epoch ANOVA showed a significant effect of epoch on end-tidal oxygen fraction [G-G $F(2.998, 270) = 92.8$, $p<0.0001$], where G-G F(adjusted degrees of freedom, numerator; adjusted degrees of freedom, denominator) indicates Greenhouse-Geisser adjustment for lack of sphericity. The interaction of gas condition and epoch was also significant [G-G $F(8.99, 270) = 13.05$, $p<0.0001$]. Repeated measures ANOVA by gas condition showed no effect of epoch for 21%O_2S [$F(9,15)=1.69$, $p>0.17$], but significant effects ($p<0.0001$) for the other three conditions [35%O_2S: $F(9, 15)=86.19$; 21%O_2F: $F(9, 14)=15.06$; 35%O_2F: $F(9, 14)=9.74$]. End-tidal PO_2 was no longer significantly different from the final value for the average of the 30 seconds from 90- to 120 seconds (120 s on Fig. 5) for 35%O_2S or for the averages of the values from 120- to 150 seconds (150 s on Fig.5) for 21%O_2F and 35%O_2F.

The $tcPO_2$ data are shown for comparison, but no statistics are presented, because the response is suspected to be partly from the conditions and partly from the instrument. (See DISCUSSION)

TCD

Visual fixation periods baseline

Baseline MCAv, used as correction factor in the analysis of changes in MCAv with phase and gas condition, was analyzed for effect of visit. Within-subject baseline MCAv values (Table 1) were not different across conditions [$F(3, 22)=0.671$, $p>0.5$].

Table 1. Baseline values, means across participants of one-minute averages of beat-to-beat measurements. Baseline was measured during breathing of 21% O_2 (air) at the start of each condition. Values in parentheses are standard deviations.

Condition	**Mean MCAv** cm/s	**Peak MCAv** cm/s	**Minimum MCAv** cm/s	**CI** cm/(s·mmHg)
21%O_2S	62 (14.6)	96 (22.6)	45 (10.6)	0.70 (0.17)
35%O_2S	63 (12.3)	96 (17.5)	46 (9.2)	0.70 (0.15)
21%O_2F	64 (13.8)	98 (18.7)	46 (10.8)	0.72 (0.18)
35%O_2F	61 (8.9)	93 (14.0)	43 (7.3)	0.68 (0.12)

Visual fixation periods time effects

Stability Measures

Even when the breathing gas was air throughout, MCAv (cm/s) decreased significantly from baseline to Phase 1 (Figure 6a), but remained stable thereafter; one-way ANOVA on phase for the 21%O_2S control condition showed a significant difference with phase [F(3,22)=3.81, p=0.024] between baseline and all the other phases; the contrast between baseline and the average of the Phases 1 to 3 was highly significant [F(1,24) =17.29, p=0.0004].

Mean arterial blood pressure (MAP), calculated as 2/3 diastolic + 1/3 systolic pressure from the brachial cuff measurements, trended upward with time (Figure 6b) independent of gas breathed. MAP showed an effect of phase only [G-G F(3, 71.8)= 23.3, p<0.0001], but no effect of gas condition [G-G F(3,70.3) = 1.09, p>0.3] and no interaction between gas and phase [F(9,210) = 0.79, p>0.6]. MAP at baseline was lower than during the conditioning phase, and that during conditioning was lower than that during Phases 2 and 3.

Heart rate (Figure 6c), like MAP, showed no significant effect of gas condition [G-G F(3,71.44) = 0.72, p>0.5] or interaction between gas condition and phase [F(9,214) = 1.0 p>0.4], but a significant effect of phase [G-G F(3,72.02] = 5.98, p<0.0001]. Heart rate during Phase 2 was significantly lower than that during all other phases, independent of the gas breathed.

We measured end-tidal CO_2 fraction ($F_{ET}CO_2$) as an indicator of CO_2 homeostasis. During Phase 2, $F_{ET}CO_2$ (Figure 6d) did not change with gas condition [for O_2 fraction, F(1,24) =2.4, p>0.1; for nested fluctuations, F(2,48)=1.03, p>0.3]. Across all phases, however, $F_{ET}CO_2$ was statistically higher at baseline (5.3%) than at other measurement times (5.2%), with an interaction also between gas condition and phase [G-G F(9, 174.1)=1.97, p=0.045]: within 21%O_2S and 35%O_2S, there were no differences between baseline and the other phases; within 21%O_2F, baseline $F_{ET}CO_2$ was higher than that during Phase 3 but not different from that during other phases; and within 35%O_2F, conditioning phase $F_{ET}CO_2$ was lower than that at baseline though not different from any of the other values. Baseline measurements were not different across gas conditions.

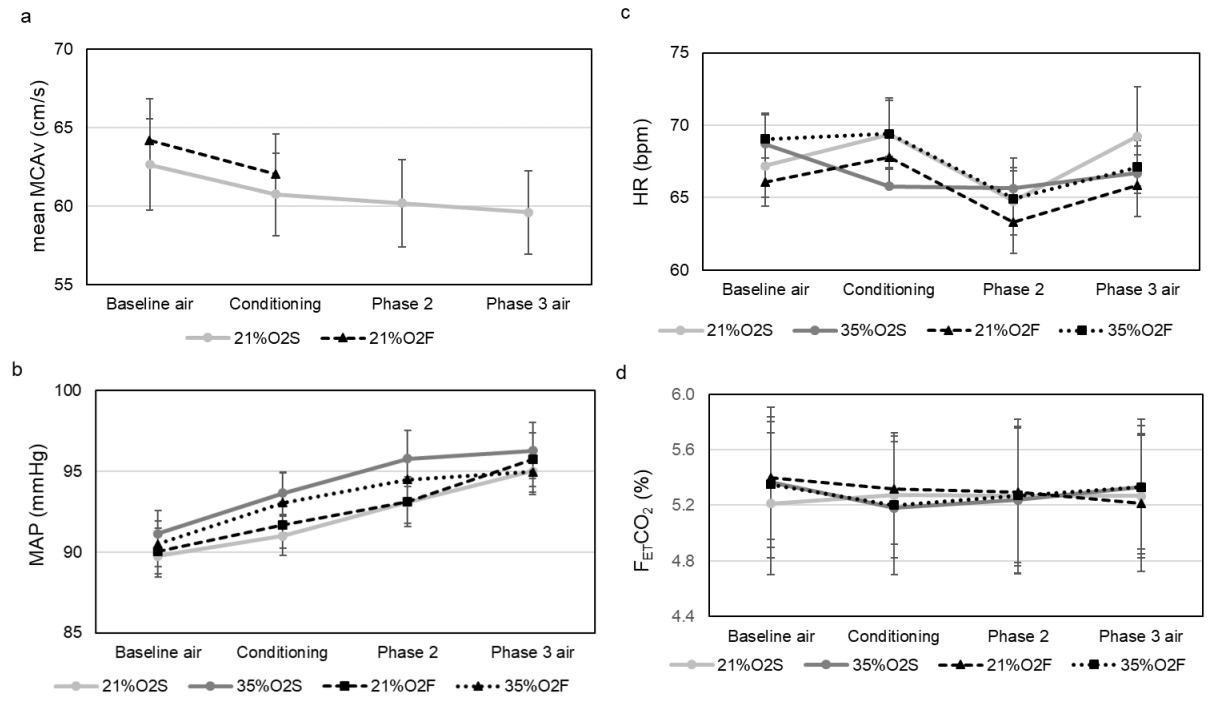

Figure 6. Effects of time during the experiment, visual fixation periods, indicators of stability, means and standard error. a) Middle cerebral artery velocity (MCAv) with uninterrupted breathing of 21% O_2, that is, all phases for 21%O_2S and the first two phases for 21% O_2F; b) Mean arterial pressure (MAP); c) heart rate; d) End tidal CO_2 fraction.

During the visual fixation periods, none of three transcranial Doppler signals considered here, MCAv, expressed as a fraction of baseline (f_MCAv) (Figure 7a), CI, also considered as fraction of baseline (f_CI) (Figure 7b), or PI (Figure 7c), varied significantly with gas condition or showed significant interaction between gas condition and phase. (Main effect of gas condition f_MCAv: $F(3,72)= 0.79$, $p>0.5$; f_CI: G-G $F(3,68.96)=0.72$, $p>0.5$; PI: $F(3,72) = 0.98$, $p>0.4$. Interaction of gas condition and phase: f_MCAv: $F(2,48)=0.52$, $p>0.6$; f_CI: G-G $F(6, 136.8)=0.83$, $p>0.5$ PI: $F(9,216)=0.60$, $p>0.7$). f_MCAv also showed no effect of phase [$F(2,48)=0.52$, $p>0.6$] However, both f_CI and PI varied with phase [f_CI: G-G $F(2, 45.82) = 7.19$, $p=0.0019$; PI: $F(3,72)=8.65$, $p<0.0001$], with f_CI decreasing between the conditioning phase and Phases 2 and 3, and PI decreasing from baseline to Phases 2 and 3.

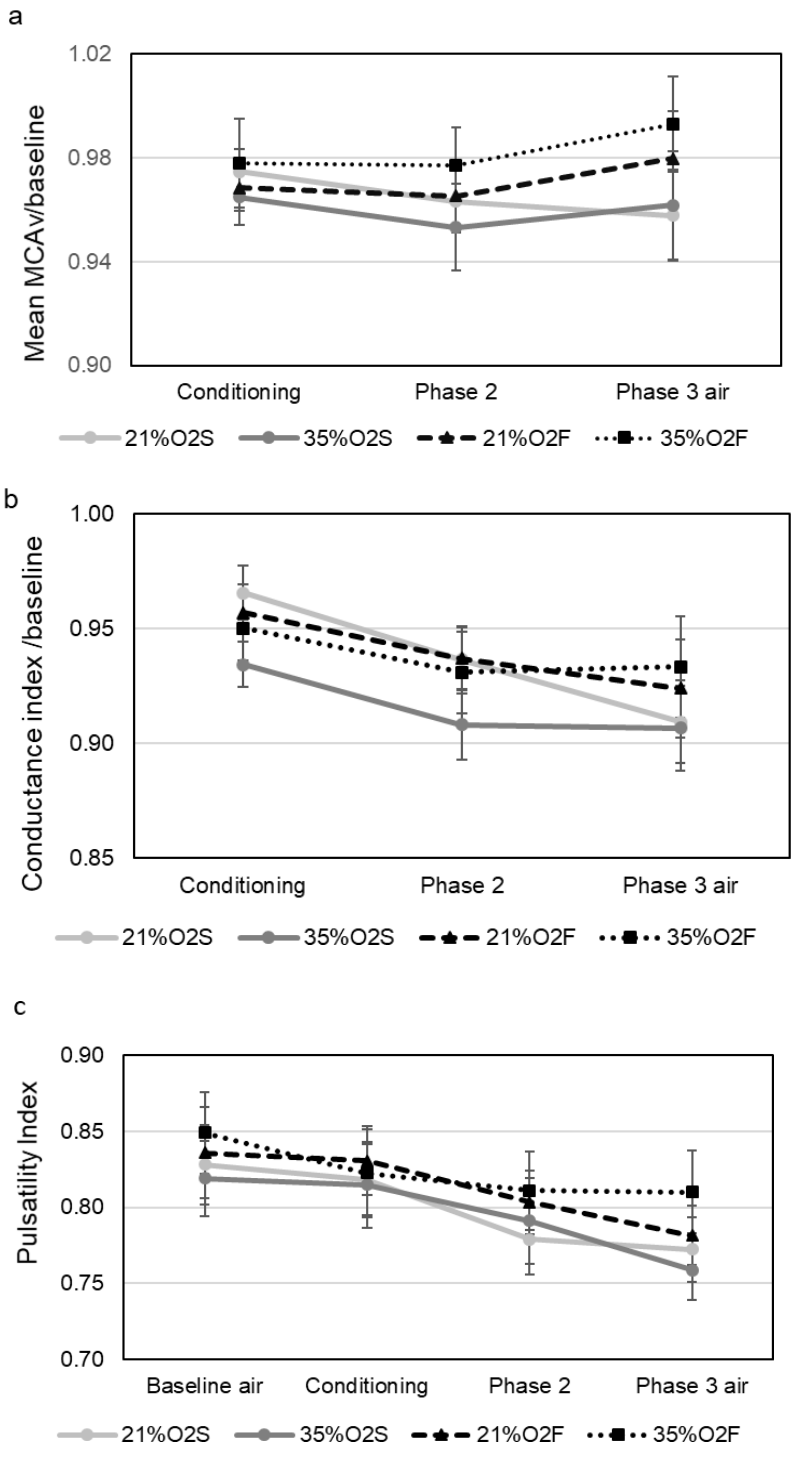

Figure 7. TCD values during visual fixation periods, mean and standard error. a) Middle cerebral artery velocity relative to baseline (f_MCAv), b) conductance index relative to baseline (f_CI), c) pulsatility index (PI).

Vigilance and Sternberg test periods

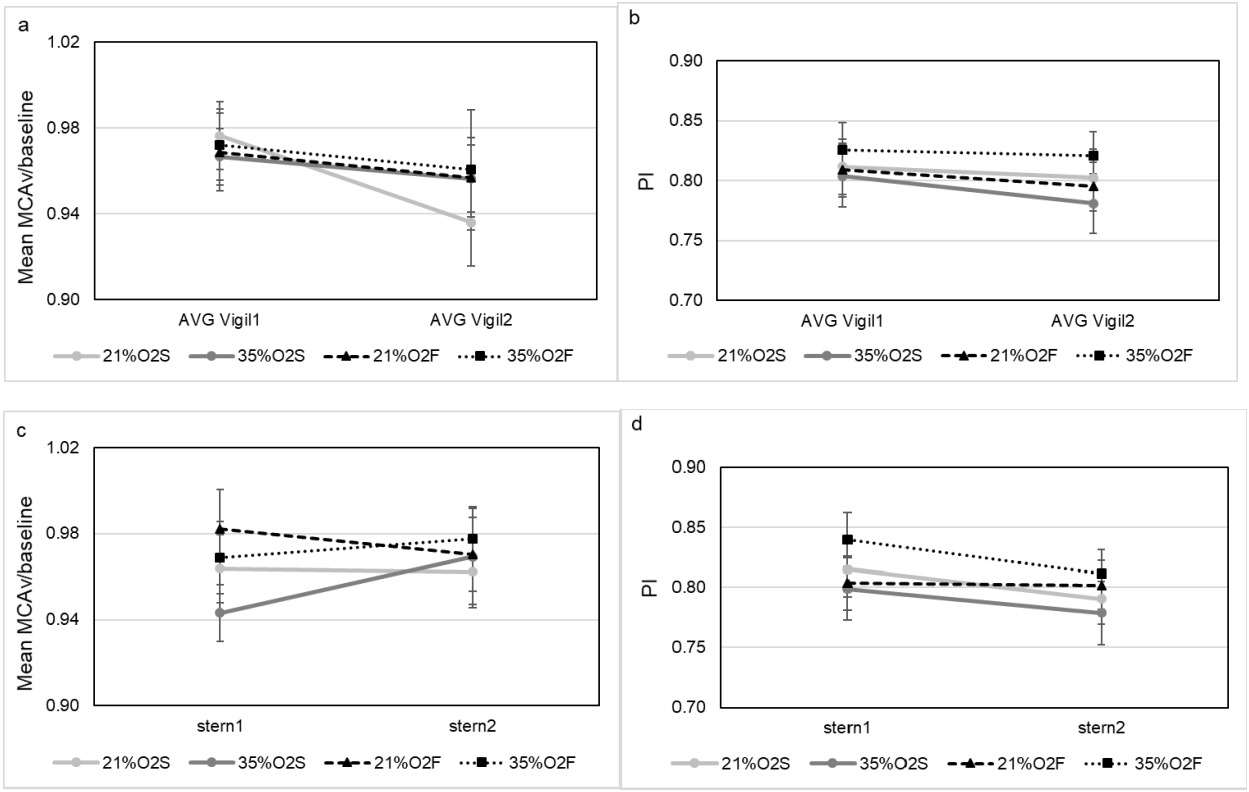

Figure 8. TCD variables during the cognitive test periods, mean and standard error a) vigilance, f_MCAv; b) vigilance, PI; c) Sternberg, f_MCAv; d) Sternberg PI. Vigil1 and Stern1, the first vigilance and Sternberg tests, were conducted in that order during Phase 2. Vigil2 began at the start of Phase 3, an air- breathing phase for all gas conditions, followed by Stern2.

Gas condition had no significant effect on MCAv (Figure 8a,c) or PI (Figure 8 c,d) during either test [Vigilance, f_MCAv: G-G $F(3, 71.42) = 0.25$, $p>0.8$; PI: G-G $F(3, 71.18) = 0.79$, $p>0.5$; Sternberg, f_MCAv: G-G $F(3, 71.4) = 0.45$, $p>0.7$; PI: G-G $F(3, 71.21) = 1.14$, $p>0.3$]. During the vigilance test, however, both MCAv and PI decreased significantly between the first and second tests [f_MCAv G-G $F(1, 20.18) = 6.09$, $p<0.023$; PI G-G $F(1,23.97) = 6.3$, $p<0.02$]. Timing of the Sternberg test did not have a significant effect on MCAv [f_MCAv G-G $F(1,24.1) = 0.77$, $p>0.3$], though PI decreased from the first to the second test [G-G $F(1, 23.95) = 13.19$, $p<0.002$]. The interaction of test timing and gas condition was not significant for the vigilance tests and either measure [test timing x gas condition f_MCAv: $F(3, 68.13) = 1.18$, $p>0.3$; PI: $F(3,71.28) = 0.98$, $p>0.4$] or for Sternberg tests and PI [G-G $F(3,71.44)=1.29$, $p>0.2$], but was marginal for the Sternberg tests and f_MCAv [G-G $F(3, 71.5)=2.1$, $p=0.107$]. The differences across periods were as great for the control measurement with 21% oxygen throughout as they were with any of the other gas conditions. Vascular conductance was not assessed because there was no measure of blood pressure during or

immediately prior to the cognitive testing.

Phase 2: fluctuations or continuation with steady gas composition

The first five minutes of Phase 2 (fluctuating gas composition for two of the four gas conditions) were analyzed in conjunction with both the starting values and ending values for the phase, that is, the values from the visual fixation periods at the ends of Phases 1 (conditioning) and 2 (fluctuations for two gas conditions) (Figure 9). Breathing gas had no significant effect, though both f_MCAv and PI showed significant differences with epoch/time [f_MCAv: G-G $F(9, 216.6)= 4.3$, $p<0.0001$; PI:G-G $F(10, 240.9) = 5.41$. $p<0.0001$], but no effects of gas condition [f_MCAv: G-G $F(3, 72.0 = 0.96$, $p > 0.4$; PI: G-G $F(3, 72.0) = 1.43$, $p>0.2$] and no significant interactions between epoch and gas [f_MCAv: $F(27, 647) = 0.9$, $p > 0.6$; PI: G-G $F(30, 719) = 0.65$; $p>0.9$].

Phase 3: air breathing

The previous breathing gas did not affect the TCD variables in the final air-breathing phase (Figure 10). In the first three minutes of Phase 3, both f_MCAv and PI showed main effects of epoch [f_MCAv: G-G $F(10, 241.6) = 4.9$, $p<0.0001$; PI: G-G $F(10, 163.9) = 5.58$, $p<0.0001$]. However, neither showed an effect of starting gas [f_MCAv:3, 72.1) = 0.20, p > 0.8; PI: G-G F(3,70.25) = 1.18, P > 0.3] or of the interaction [f_MCAv: $F(30, 712.7) = 1.02$, $p>0.43$; PI: G-G $F(30, 618.5) = 1.09$, $p>0.3$].

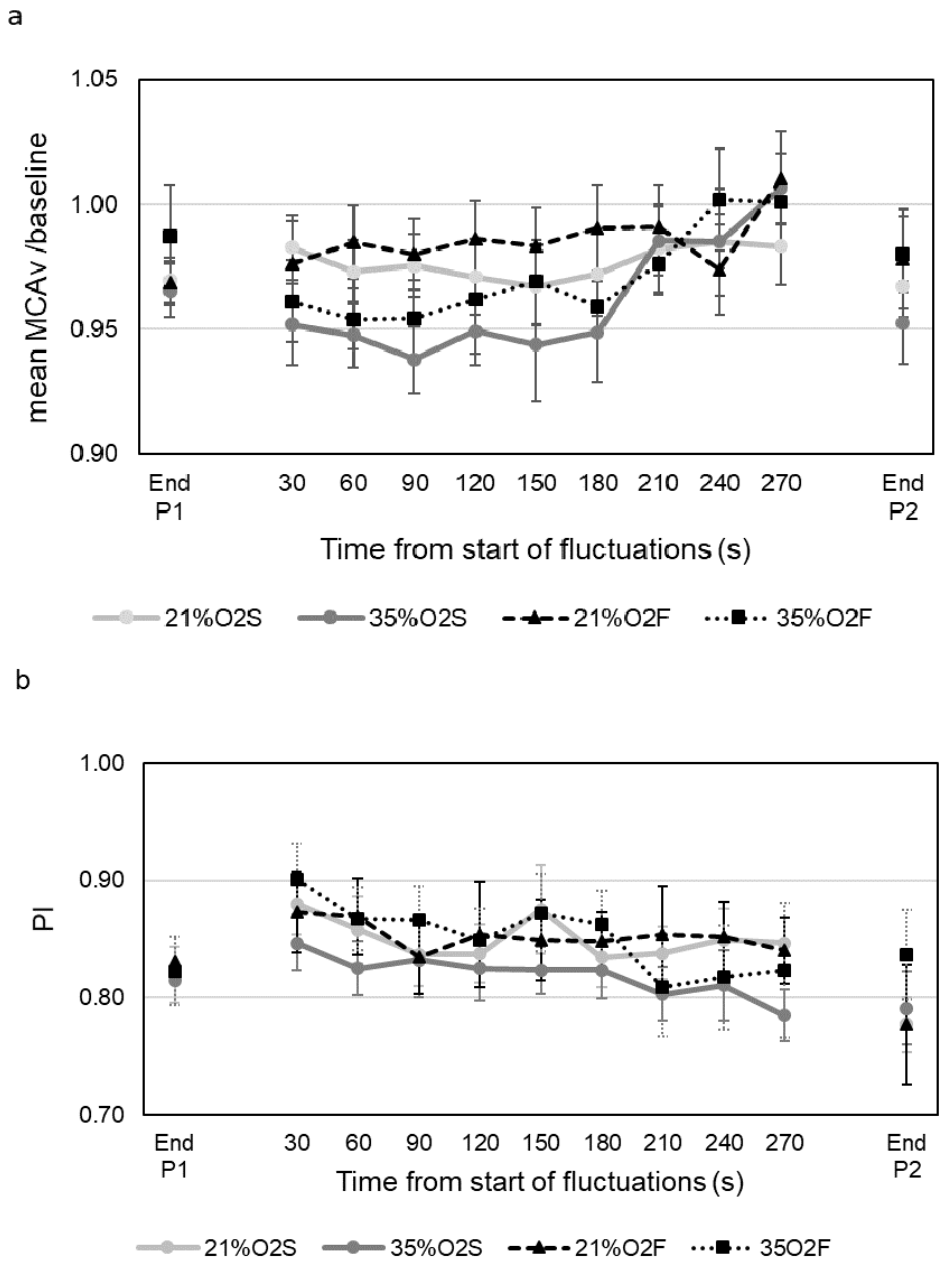

Figure 9. TCD variables averaged across participants at the start of Phase 2, means and standard errors. a) Mean MCAv expressed as fraction of baseline (f_MCAv) and b) PI. Connected points represent the averages of 20 second (fluctuations) or 30 second (steady gas) of data for each participant. The first and last points are one minute averages.

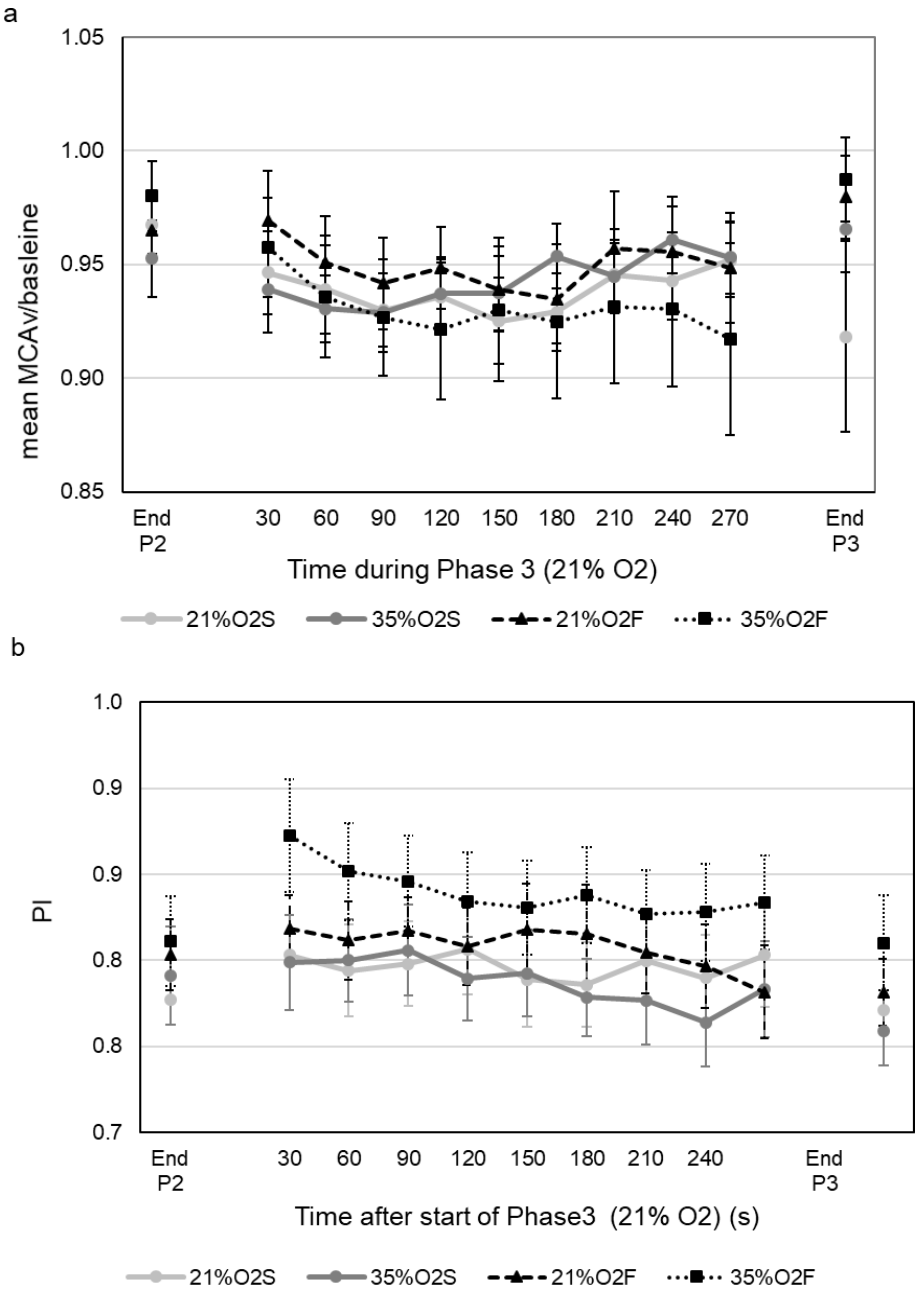

Figure 10. TCD variables averaged across participants at the start of Phase 3, means and standard errors, a) f_MCAv, b) PI. Connected points represent the average of 30 seconds of data for each participant. The first and last points are one minute averages.

NIRS

All results for NIRS data can be seen in Figure 11 with separate panels for visual fixation periods, the vigilance task, the Sternberg task, and Phase 2. Separate plots depict relative changes in HHb and O_2Hb concentrations.

Visual fixation periods

Visual fixation period data were analyzed as a 4 (condition: 21%O_2S, 21%O_2F, 35%O_2S, 35%O_2F) x 4 (phase: baseline, P1, P2, P3) repeated-measures ANOVA.

For HHb, both the main effect of condition, $F(3,21)=7.820$, $p<0.001$, $\eta_p^2=0.254$, and of phase, $F(3,21)=40.245$, $p<0.001$, $\eta_p^2=0.636$, were significant. Importantly, the condition x phase interaction was also significant, $F(9,15)=17.813$, $p<0.001$, $\eta_p^2=0.436$. To follow-up on this interaction, separate one-way ANOVAs on condition were tested for each phase. For the baseline phase, the main effect of condition was non-significant, $p=0.283$, which is expected because participants breathed 21% oxygen during the baseline regardless of the condition. During P1, there was a significant main effect of condition, $F(3,21)=27.825$, $p<0.001$, $\eta_p^2=0.547$, whereby there was a significant reduction in HHb concentration during 35%O_2S, 35%O_2F compared to 21%O_2S and 21%O_2F, all $p<0.001$. During P2, the main effect of condition was also significant, $F(3,21)=12.837$, $p<0.001$, $\eta_p^2=0.358$, whereby there was a significant reduction in HHb for the 35%O_2S and both + flux conditions compared to 21%O_2S, all $p<0.01$. During P3, the main effect of condition was no longer significant, $F(3,21)=0.519$, $p=0.671$.

For O_2Hb, the main effect of condition was not significant, $F(3,21)=1.223$, $p=0.308$, but the main effect of phase was significant, $F(3,21)=19.292$, $p<0.001$, $\eta_p^2=0.456$. Also, the condition x phase interaction did reach significance, $F(9,15)=2.218$, $p=0.022$, $\eta_p^2=0.088$. To follow-up on the significant interaction, separate one-way ANOVAs on condition were tested for each phase. All four phases yielded non-significant main effects of condition, all $p\geq0.129$.

Taken together, these results demonstrate that there was a significant reduction in HHb when participants breathed 35% O_2 compared to when they breathed 21% O_2. In line with that, HHb remained at an intermediary level when fluctuating between 21% and 35% compared to the two constant conditions. Participants' HHb returned to baseline levels during the recovery phase. While O_2Hb data showed a similar pattern in the opposite direction, the effects were less robust than the HHb results, but generally breathing 35% O_2 in a constant or fluctuating context increased O_2Hb.

Vigilance task

The vigilance task was completed during P2 and P3. Vigilance task data were therefore analyzed using a 4 (condition: 21%O_2S, 21%O_2F, 35%O_2S, 35%O_2F) x 2 (phase: P2, P3) repeated-measures ANOVA.

For HHb, both the main effect of condition, $F(3,21)=7.779$, $p<0.001$, $\eta_p^2=0.253$, and

phase, $F(1,23)=31.801$, $p<0.001$, $\eta_p^2=0.580$, were significant. In addition, the condition x phase interaction was significant, $F(3,21)=17.822$, $p<0.001$, $\eta_p^2=0.437$. To follow-up on this interaction, separate one-way ANOVAs on condition were tested for each phase. For P2, the main effect of condition was significant, $F(3,21)=11.244$, $p=0.003$, $\eta_p^2=0.328$, whereby there was a significant reduction in HHb for the 35%O_2S and both flux conditions compared to 21%O_2S, all ps<0.005. Further, there was a significant reduction in HHb for 35%O_2S compared to 21%O_2F, $p=0.009$. For P3, the main effect of condition was not significant, $F(3,21)=1.580$, $p=0.202$.

For O_2Hb, the main effect of phase was significant, $F(1,23)=39.833$, $p<0.001$, $\eta_p^2=0.634$. However, neither the main effect of condition, $F(3,21)=0.802$, $p=0.497$, nor the condition x phase interaction, $F(3,21)=1.279$, $p=0.288$, reached significance.

Sternberg task

The Sternberg memory task was completed during P2 and P3, therefore data were analyzed using a 4 (condition: 21%O_2S, 21%O_2F, 35%O_2S, 35%O_2F) x 2 (phase: P2, P3)35%O2F repeated-measures ANOVA.

For HHb, both the main effect of condition, $F(3,21)=4.908$, $p=0.004$, $\eta_p^2=0.176$, and phase, $F(1,23)=77.410$, $p<0.001$, $\eta_p^2=0.771$, were significant. In addition, the condition x phase interaction was significant, $F(3,21)=23.028$, $p<0.001$, $\eta_p^2=0.500$. To follow-up on this interaction, separate one-way ANOVAs on condition were tested for each phase. For P2, the main effect of condition was significant, $F(3,21)=15.432$, $p<0.001$, $\eta_p^2=0.402$, whereby there was a significant reduction in HHb for the 35%O_2S and both flux conditions compared to 21%O_2S, all $p<0.005$. Further, there was a significant reduction in HHb for 35%O2S compared to the 21% + flux condition, $p=0.040$. For the recovery phase, the main effect of condition was not significant, $F(3,21)=0.408$, $p=0.748$.

For O_2Hb, the main effect of phase was significant, $F(1,23)=83.643$, $p<0.001$, $\eta_p^2=0.784$. However, neither the main effect of condition, $F(3,21)=1.614$, $p=0.194$, nor the condition x phase interaction, $F(3,21)=1.653$, $p=0.185$, reached significance.

Both the vigilance and Sternberg task periods showed a similar pattern of NIRS results. During P2, HHb significantly decreased in the 35%O_2S condition and was at an intermediary level for the 21%O_2F and 35%O_2F conditions compared to 21%O_2S. Notably, HHb levels returned to baseline levels during P3. For O_2Hb, again the reverse pattern (i.e., increased O_2Hb when breathing 35%) was present but did reach the same level of significance as the HHb results.

Phase 2: fluctuations or continuation with steady gas composition

Data from the first 5 minutes of P2 were examined to see how quickly a change in HHb or O_2Hb occurred under the fluctuating conditions. A 4 (condition: 21%O_2S, 21%O_2F, 35%O_2S, 35%O_2F) x 5 (one-minute epochs) repeated-measures ANOVA was tested.

For HHb, both the main effect of condition, $F(3,20)=11.740$, $p<0.001$, $\eta_p^2=0.348$, and of epoch, $F(4,19)=7.596$, $p<0.001$, $\eta_p^2=0.257$, were significant. Additionally, the condition x epoch interaction was significant, $F(12,11)=7.110$, $p<0.001$, $\eta_p^2=0.244$. To follow-up on this interaction, separate one-way ANOVAs on condition were tested for each epoch. All 5 one-minute epochs showed a significant main effect of condition, all $p<0.001$. During epochs 1 and 2, there was a significant reduction in HHb for the 35%O_2S and 35%O_2F conditions compared to the 21%O_2S and 21%O_2F conditions, both $p<0.05$. During epochs 3 and 4, there was a significant reduction in HHb for 35%O_2S and 35%O_2F compared only to 21%O_2S, both $p<0.05$. For epoch 5, there was a significant reduction in HHb for 35%O_2S and 21%O_2F compared to 21%O_2S, all $p<0.05$, and a trend for a difference between 35%O_2F and 21%O_2S, $p=0.056$. Further, the minute 5 difference between 21%O_2F and 35%O_2S trended toward significance as well, $p=0.059$.

For O_2Hb, the main effect of epoch was significant, $F(4,19)=15.915$, $p<0.001$, $\eta_p^2=0.420$. However, neither the main effect of condition, $F(3,20)=1.272$, $p=0.291$, nor the condition x phase interaction, $F(12,11)=0.708$, $p=0.743$, reached significance.

Within the first 5 minutes of P2, a gradual change in HHb was observed, whereby the preceding P1 oxygen concentration exerted its effects for the first 2-3 minutes, then values leveled off. By the end of the 5- minute analysis period, the expected changes, as noted above in the rest periods and during the cognitive tasks, had emerged. Namely, a decrease in HHb when breathing 35% oxygen and an intermediary level for the 21%O_2F and 35%O2F conditions compared to 21%O_2S. Again, O_2Hb showed a similar pattern, albeit opposite polarity, but to a lesser degree.

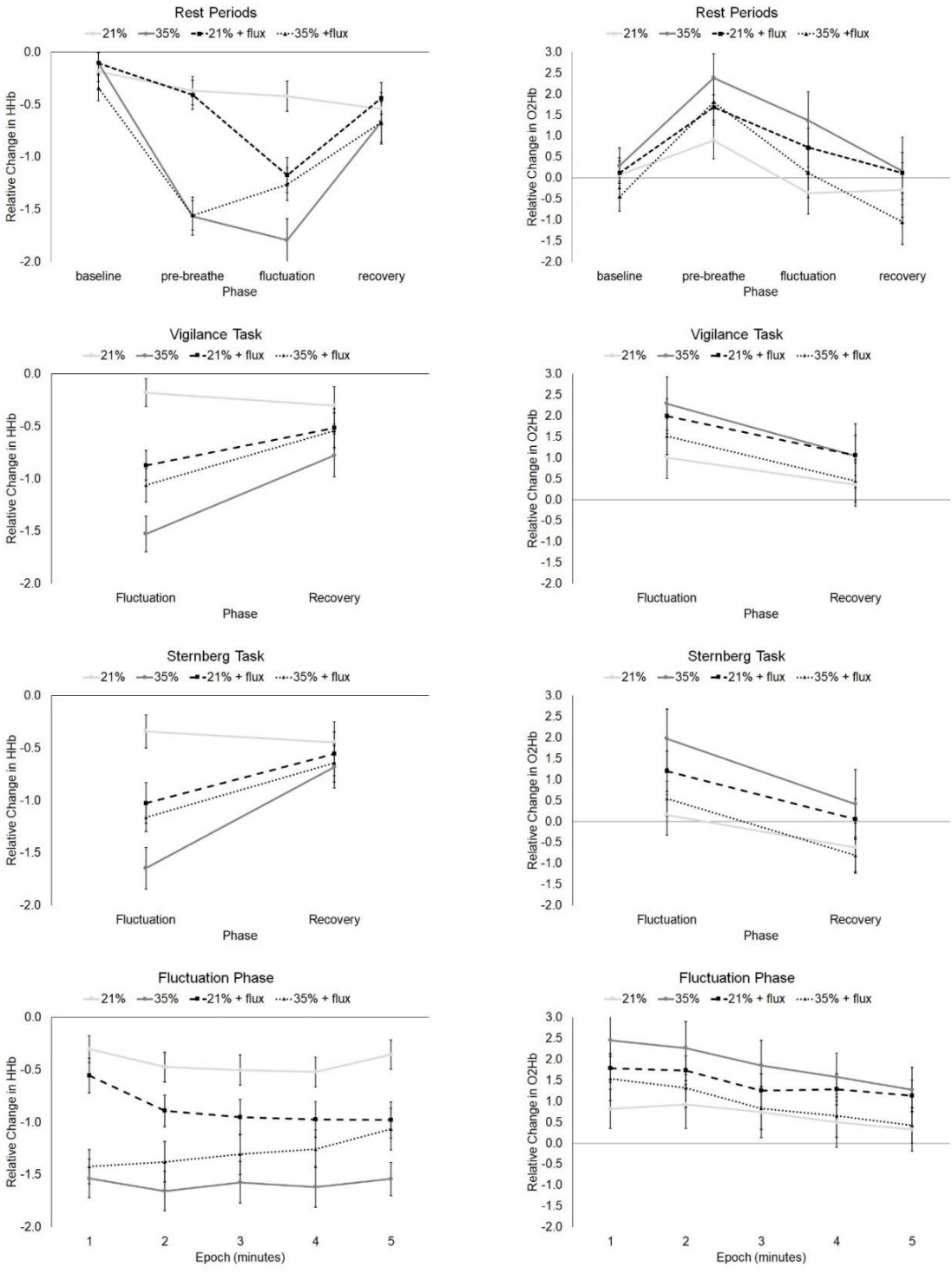

Figure 11. NIRS data shown for the rest (i.e., visual fixation) periods, the vigilance task, the Sternberg task, and the first 5 minutes of P2. Data are shown by condition and separate plots illustrate HHb changes (left column) and O_2Hb changes (right column). Error bars represent standard error of the mean.

Cognitive results and questionnaire responses

Sternberg task performance

One participant was excluded from analyses due to accuracy at or near 0% for multiple time points. For the remaining 24 participants, accuracy was tested using a 4 (condition: 21%O_2S, 21%O_2F, 35% O_2S, 35% O_2F) x 2 (phase: fluctuation, recovery) repeated measures ANOVA. Neither the main effect of condition, $F(3,21)=0.157$, $p=0.925$, nor the main effect of phase, $F(1,23)=0.688$, $p=0.415$, was significant. Likewise, the condition x phase interaction was not significant, $F(3,21)=0.152$, $p=0.928$. For response time (RT), a 4 (condition: 21%O_2S, 21%O_2F, 35% O_2S, 35% O_2F) x 2 (phase: P2, P3) repeated measures ANOVA was also used. RT was calculated based only on correct trials. Neither the main effect of condition, $F(3,21)=0.736$, $p=0.534$, nor the main effect of phase, $F(1,23)=1.842$, $p=0.188$, was significant. Similarly, the condition x phase interaction was not significant, $F(3,21)=0.984$, $p=0.406$. Results for both accuracy and RT are shown in Figure 12. Together these results suggest that our experimental manipulation of breathing 21% versus 35% versus fluctuating between the two did not impact short-term memory performance.

Vigilance task performance

Repeated measures ANOVAs, 4 (condition: 21%O_2S, 21%O_2F, 35%O_2S, 35%O_2F) x 2 (phase: P2, P3) were used for several aspects of the vigilance Task. For critical trial accuracy, the main effect of condition approached but did not reach significance, $F(3,22)=2.515$, $p=0.065$. Both the main effect of phase, $F(1,24)=0.673$, $p=0.420$, the condition x phase interaction, $F(3,22)=1.341$, $p=0.268$ failed to reach significance. Post-hoc analysis revealed that there was a significant difference in critical trial accuracy between 21%O_2S and 35%O_2F, $p=0.027$, and between 21%O_2F and 35%O_2F, $p=0.045$. However, those comparisons do not survive multiple comparisons correction.

For neutral trial accuracy, neither the main effect of condition, $F(3,22)=0.819$, $p=0.488$, nor the main effect of phase, $F(1,24)=0.146$, $p=0.706$, approached significance. In addition, the condition x phase interaction was non-significant, $F(3,22)=0.997$, $p=0.400$.

Finally for critical trial RT, correct trials only, neither the main effect of condition, $F(3,22)=1.735$, $p=0.168$, nor the main effect of phase, $F(1,24)=2.148$, $p=0.156$, approached significance. In addition, the condition x phase interaction was non-significant, $F(3,22)=1.037$, $p=0.381$. Results for accuracy and RT are shown in Figure 12. Together these results suggest that our experimental manipulation of breathing 21% versus 35% constant oxygen versus fluctuating between the two did not impact sustained attention performance.

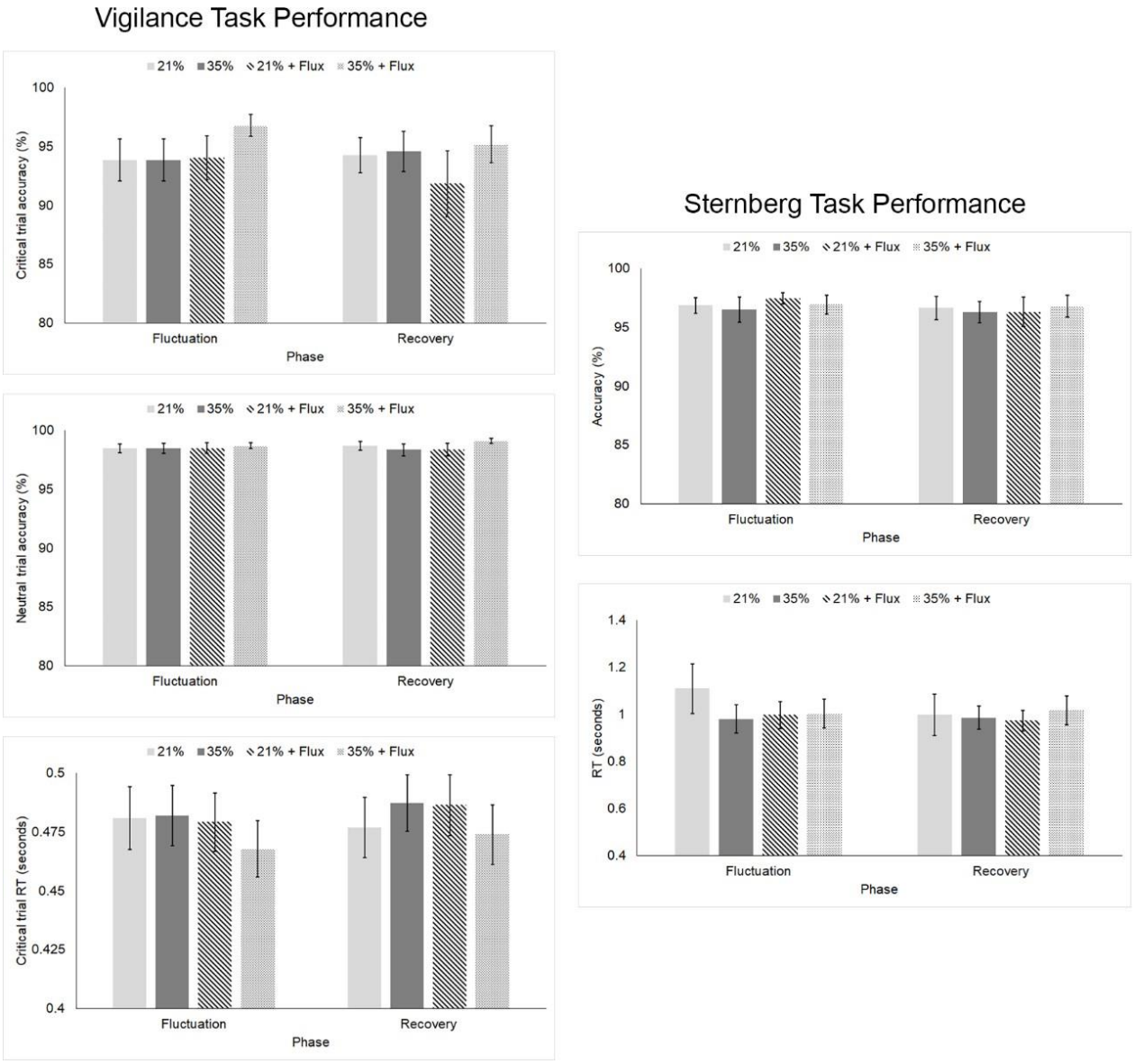

Figure 12. Vigilance and Sternberg task accuracy and RT results by condition and phase. Error bars represent standard error of the mean.

Environmental Symptoms Questionnaire (ESQ)

The ESQ was administered at three phases: prior to the baseline period (i.e., pre-baseline), during P2, and during P3. The questionnaire is shown in the Appendix. For each sub-scale of the ESQ, a 4 (condition: 21%O_2S, 21%O_2F, 35%O_2S, 35%O_2F) x 3 (phase: pre-baseline, fluctuation, recovery) repeated-measures ANOVA was tested. Results are illustrated in Figure 13. Significant main effects were followed up with planned post-hoc comparisons, corrected for multiple comparisons with a Šidák correction.

Cerebral Subscale. For the cerebral sub-scale, the main effect of phase was significant, F(2,23)=13.327, p<0.001, ηp2=0.357. Post-hoc comparisons demonstrated that participants reported more cerebral symptoms during P2 compared to pre-baseline, p=0.003. In addition, participants reported more cerebral symptoms during P3 compared to pre-baseline, p=0.002. There was no difference between the P2 and P3 phases, p=0.155. Neither the main effect of condition, F(3,22)=0.323, p=0.809, nor the condition x phase interaction, F(6,19)=0.532, p=0.783, approached significance.

Respiratory Subscale. For the respiratory sub-scale, the main effect of phase was significant, $F(2,23)=12.755$, $p<0.001$, $\eta_p^2=0.347$. Post-hoc comparisons demonstrated that participants reported more respiratory symptoms during P2 compared to pre-baseline, $p=0.005$. In addition, participants reported more respiratory symptoms during P3 compared to pre-baseline, $p=0.003$ and more symptoms during P3 compared to P2, $p=0.043$. Neither the main effect of condition, $F(3,22)=0.897$, $p=0.447$, nor the condition x phase interaction, $F(6,19)=0.294$, $p=0.939$, approached significance.

Ear, Nose, and Throat Subscale. For the ENT sub-scale, the main effect of phase was significant, $F(2,23)=19.132$, $p<0.001$, $\eta_p^2=0.444$. Post-hoc comparisons demonstrated that participants reported more ENT symptoms during P2 compared to pre-baseline, $p<0.001$. In addition, participants reported more ENT symptoms during P3 compared to pre-baseline, $p<0.001$. There was no difference between P2 and P3, $p=0.633$. Neither the main effect of condition, $F(3,22)=0.593$, $p=0.622$, nor the condition x phase interaction, $F(6,19)=1.141$, $p=0.342$, approached significance.

*Cold Subscale***.** For the cold sub-scale, the main effect of phase was significant, $F(2,23)=10.654$, $p<0.001$, $\eta_p^2=0.307$. Post-hoc comparisons demonstrated that participants reported more cold symptoms during P2 compared to pre-baseline, $p=0.013$. In addition, participants reported more cold symptoms during P3 compared to pre-baseline, $p=0.007$ and more symptoms during P3 compared to P2, $p=0.042$. The main effect of condition was not significant, $F(3,22)=1.736$, $p=0.167$. However, the condition x phase interaction, $F(6,19)=2.266$, $p=0.040$, was significant. This interaction appears to be driven by a decrease in symptoms reported during the 21%O_2F condition during fluctuation and recovery phases compared to the other three conditions.

Distress Subscale. For the distress sub-scale, the main effect of phase was significant, $F(2,23)=16.496$, $p<0.001$, $\eta_p^2=0.407$. Post-hoc comparisons demonstrated that participants reported more distress symptoms during P2 compared to pre-baseline, $p<0.001$. In addition, participants reported more distress symptoms during P3 compared to pre-baseline, $p<0.001$. There was no difference between P2 and P3, $p=0.985$. Neither the main effect of condition, $F(3,22)=2.066$, $p=0.112$, nor the condition x phase interaction, $F(6,19)=1.041$, $p=0.401$, approached significance.

Alert Subscale. For the alert sub-scale, the main effect of phase was significant, $F(2,23)=12.332$, $p<0.001$, $\eta_p^2=0.339$. Post-hoc comparisons demonstrated that participants reported being less alert during P2 compared to pre-baseline, $p=0.003$. In addition, participants reported being less alert during P3 compared to pre-baseline, $p=0.002$. There was no difference between P2 and P3, $p=0.999$. Additionally, the main

effect of condition was significant, $F(3,22)=3.567$, $p=0.018$, $\eta_p^2=0.129$. Post-hoc comparisons demonstrated that participants reported being more alert in the 35%O_2F condition compared to the 21%O_2F condition, $p=0.037$. The condition x phase interaction did not reach significance, $F(6,19)=1.231$, $p=0.294$.

Exert Subscale. For the exert sub-scale, the main effect of phase was significant, $F(2,23)=10.309$, $p<0.001$, $\eta_p^2=0.300$. Post-hoc comparisons demonstrated that participants reported more exertion symptoms during P2 compared to pre-baseline, $p=0.022$. In addition, participants reported more exertion symptoms during P3 compared to pre-baseline, $p=0.003$. There was no difference between P2 and P3, $p=0.297$. Neither the main effect of condition, $F(3,22)=0.123$, $p=0.946$, nor the condition x phase interaction, $F(6,19)=0.218$, $p=0.971$, approached significance.

*Muscle Subscale***.** For the muscle sub-scale, the main effect of phase was significant, $F(2,23)=21.043$, $p<0.001$, $\eta_p^2=0.467$. Post-hoc comparisons demonstrated that participants reported more muscle symptoms during P2 compared to pre-baseline, $p<0.001$. In addition, participants reported more muscle symptoms during P3 compared to pre-baseline, $p<0.001$ and more symptoms during P3 compared to P2, $p=0.011$. Neither the main effect of condition, $F(3,22)=0.491$, $p=0.690$, nor the condition x phase interaction, $F(6,19)=1.201$, $p=0.309$, approached significance.

Fatigue Subscale. For the fatigue sub-scale, neither the main effect of phase, $F(2,23)=3.093$, $p=0.054$, nor the main effect of condition, $F(3,22)=0.298$, $p=0.826$, reached significance. The condition x phase interaction was also not significant, $F(6,19)=2.099$, $p=0.057$.

The results from the ESQ sub-scales collectively demonstrate a change in symptom reporting by study phase, but not by condition. Therefore, the effects cannot be attributed to changes in the gas mixture being breathed. Instead, participants appeared to report an increased rate of symptoms as the data collection period wore on. Participants were seated in a constrained position while wearing the equipment for nearly 90 minutes. We would interpret the increased symptom reporting over time as indicative of participants' growing discomfort. Also noteworthy is that most of the average ratings fell between 0 (i.e., not at all) and 1 (i.e., slight), with only Alert and Fatigue sub-scales averaging closer to 2 (Figure 13).

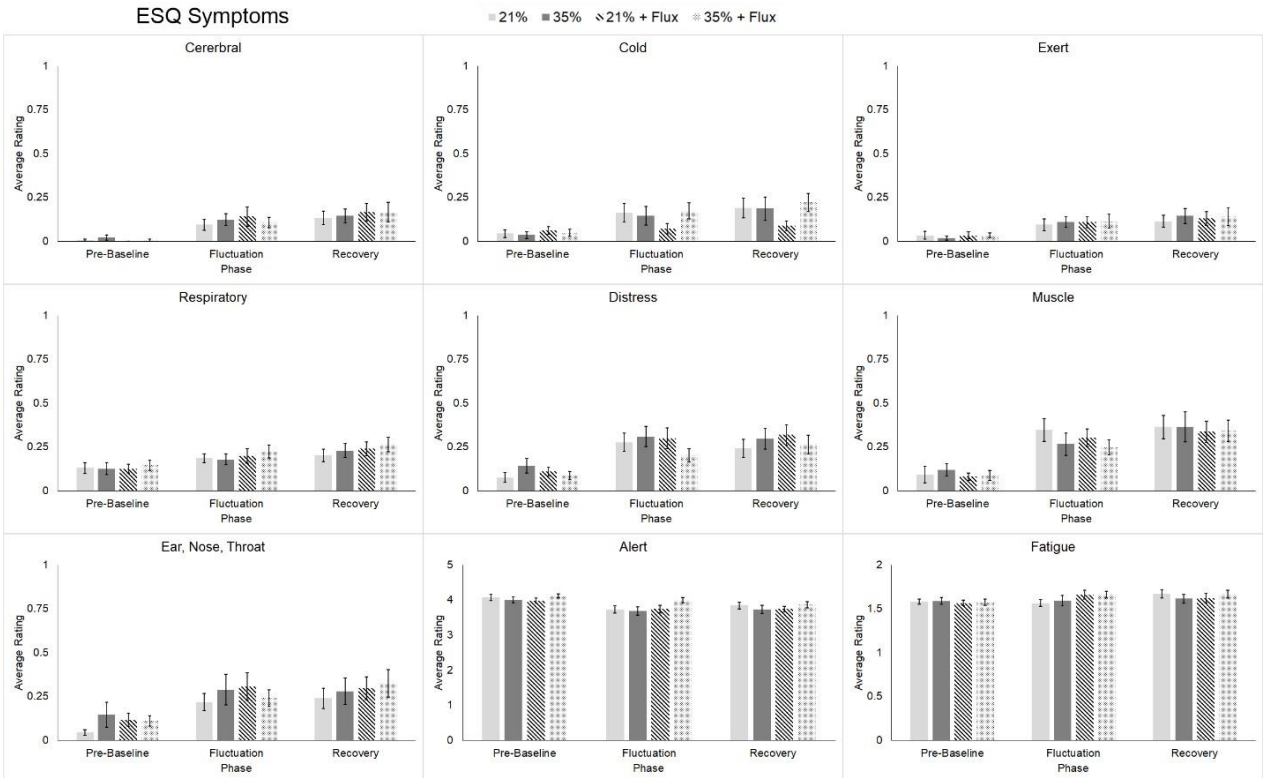

Figure 13. ESQ data for each sub-scale, shown for each condition and phase. Error bars represent standard error of the mean.

DISCUSSION

The principal findings of this study were that neither steady breathing of 35% O_2 for 50 minutes, nor fluctuations in inhaled oxygen percentage between 21% and 35% with 30-seconds per gas, caused significant changes in middle cerebral artery flow velocity as measured using TCD. Return to 21% O_2 was equally uneventful. Similarly, there was no decrease in oxygenation in the frontal cortex as measured by NIRS. There was a consistent decrease in frontal cortex HHb when participants breathed steady 35% or fluctuated between 21% and 35%, but this was anticipated. Vigilance and short-term memory performance were not impaired or facilitated, and no consistent set of symptoms was reported. To extrapolate these findings to equivalent oxygen concentrations in tactical aircraft, fluctuations with the gas changing every 30 seconds between30% and 45% O_2 at a cabin altitude of 8,000 ft MSL would not appear to affect oxygen delivery to the brain.

Gas mixing

Gas-switching with the valves very close to the mouthpiece provided clean gas steps with minimal mixing; inspired gas changed step-wise. Thus, the changes in arterial oxygen partial pressure in this experiment were as rapid as is physically possible in a resting person who is breathing normally. Even if the gas composition at the OBOGS gas concentrator output were to change step-wise, the perturbations that reached to mask would be more gradual; step changes in composition in a flowing system are smoothed by axial dispersion even without turbulence or a mixing chamber (plenum) in the system (10).

The measured changes in end-tidal oxygen fraction were both more gradual and lower in amplitude than were the imposed fluctuations in inspired oxygen fraction. End-tidal gas, the gas exhaled at the end of a breath, is a sample of alveolar gas. Alveolar gas composition changes by serial dilution, one breath at a time. The lung gas always present at the end of an expiration, approximately 3 L in adults, transforms a step change in inhaled gas to an exponential rise in alveolar gas, with the speed of the dilution depending on the size of the breath (tidal volume) and the number of breaths per minute (breathing frequency). The start of Phase 3 indicates that a step change in gas composition must last more than 90 seconds for its full effect to be manifest in the lungs of a person at rest and breathing spontaneously; of course, a gas composition change could have physiological consequences before the transition was complete if, for example, the second gas contained no oxygen. At the start of Phase 2, after 2- to 3 minutes the conditioning gas no longer mattered; the 30-second gas fluctuations resulted in small fluctuations around an alveolar value that was the average of the steady-state value for the two inspired gases.

Although alveolar gas composition varies somewhat within the lungs and from end inspiration to end expiration, end-tidal gas is an indicator of the gas partial pressures in pulmonary capillary blood. Arterial PO_2 is the volume-weighted average of all capillary PO_2. Thus, arterial PO_2 during the gas fluctuations, the PO_2 delivered to the brain at that

time, had fluctuations similar to or slightly slower than those of the end-tidal oxygen fraction. The settling time to the new steady state end-tidal gas was reflected in the NIRS data, which also reached a new stable value in 2 to 3 minutes.

The changes in $tcPO_2$ were smoother and slower than those in the alveolar gas, but also slower than those in the NIRS data for oxygenation in the frontal region of the brain. With return to 21% O_2 as breathing gas, $tcPO_2$ reached its new value after approximately five minutes, matching the step change response (delay plus 90% response time of more than 5 minutes) reported by others (11). For both this experiment and the published value, the response was measured from a change in inhaled gas to a change in $tcPO_2$, encompassing the gas mixing time, circulatory time, and instrument response time. The quantity of interest is the time for gas mixing and circulatory response. Because the $tcPO_2$ response was slower than the NIRS response, the instrument response time, including diffusion through the skin and into the sensor element, appears to be slow enough to blunt the measurement. Transcutaneous measurements can be used reliably only for the steady- and slowly-changing conditions for which they were developed.

Doppler ultrasound

The Doppler Effect is the name given the fact that relative motion between the source of a wave and an object from which the wave is reflected alters the wavelength. The wavelength of the reflection becomes shorter if the object approaches the source, and longer if the object moves away. Because frequency is inversely proportional to wavelength, the change in frequency of the sound can thus be used to measure the velocity of the object targeted. For Doppler ultrasound blood velocity measurement, the target objects are red blood cells. Sound reflects from the red cells, which have different velocities depending on their location relative to the wall of the blood vessel under insonation. The distribution of velocities across the sampling volume at each sampling time is displayed as a velocity waveform. Maximum, minimum, and mean velocity can be extracted for each heartbeat.

The frequency shift gives a true measure of velocity only if the ultrasound beam is parallel to the direction of flow; the values are lower than true velocity by a factor equal to the cosine of any deviation from parallel. The angle between the ultrasound beam and the flowing blood is slightly different each time a Doppler probe is placed. To remove the confounder of probe angle which indubitably differed among participant visits, MCAv was assessed as fraction of baseline for the session. The lack of statistical difference of the baseline values across gas condition (visit) indicates that the measurements were consistent, but the elimination of the angle differences increases the precision of the analyses.

Measurements - interpretation of variables

We measured flow velocity, but the true variable of interest is volumetric flow. If cerebral blood flow is constant, any increase in mean velocity indicates a decrease in cross-sectional area, that is, constriction, of the blood vessel. In healthy populations, cerebral autoregulation is presumed to hold mean brain blood flow constant in the face of variations in blood pressure. Thus, changes in MCAv are generally interpreted as changes in cross-sectional area. However, hypercapnia (12) and hypoxia (13, 14) increase the blood flow at which the cerebral vasculature self-regulates, and hypocapnia (12) and hyperoxia (15) decrease cerebral blood flow. With alterations in breathing gas, changes in cerebral flow are caused by changes in resistance in the cerebral circulation, at least in part through changes in the diameter of the MCA (14). Thus, when arterial blood gases are manipulated, neither mean cerebral blood flow nor vascular diameter can be assumed to be constant. If both radius and flow change, velocity can increase or decrease depending on the relative changes of the two. However, if the decrease in flow is presumed to be caused by local vasoconstriction and flow is presumed to be proportional to the fourth power of the radius (Poiseuille flow), a decrease in flow leads to a decrease in velocity. In this experiment, both are reasonable assumptions, and decreased MCAv would have been interpreted as decreased volumetric flow. The lack of change in MCAv thus implies a lack of change volumetric flow.

Vascular conductance is defined as the ratio of the volumetric flow to the pressure difference between the arterial inlet and the relevant venous circulation. Mean arterial pressure (MAP) is the average driving pressure between the large arteries and the central veins where pressure is approximately ambient, not the pressure across the cerebral circulation. The pressure drop is minor in the large arteries, but the upstream arterial geometry and the presence of resistance components decrease the pressure in the MCA; for example, in normotensive individuals at rest, arterial pressure in the MCA is approximately 15 mmHg lower than that in the brachial artery (16). Downstream, the pressure difference from normal cerebral venous circulation to the great veins may have little influence on flow; in standing individuals, mean MCAv during Valsalva maneuvers relates more to arterial pressure than to central venous pressure (17). The venous pressure in our experiments can be assumed close enough to 0 mmHg relative to ambient pressure that it can be ignored. This would not be the case if intracranial pressure were elevated (18), or if central venous pressure elevation caused an increase in venous pressure in the neck (17). However, under the conditions of this experiment, MAP represented the mean driving pressure for blood flow from the carotid artery. Changes in conductance index caused by an intervention relate to effects of the intervention on the entire circulation rather than changes specific to the MCA.

The usual index of cerebrovascular vascular conductance, CI = MCAv/MAP (19), assumes constant vessel diameter to substitute MCA velocity for volumetric flow (velocity multiplied by cross-sectional area). If flow changes because of vasoconstriction at constant driving pressure in a Poiseuille flow regime, flow is proportional to radius to the fourth power, as described above, while velocity is proportional to radius squared. In the presence of vasoconstriction, the index of conductance calculated using velocity

rather than flow will underestimate any decreases in conductance. This is somewhat moot, because we saw no significant changes in conductance index.

Conductance is the inverse of resistance. Gosling's pulsatility index (PI), the ratio of the peak-to-peak amplitude of the velocity waveform to the mean velocity, is often used as an index (not a direct measurement) of resistance. PI has normal values between 0.5 and 1.2 (20). When the cerebral venous pressure is normal, increases in arterial pulse pressure, heart rate, resistance, or blood vessel compliance at or downstream of the measurement point increase PI (21). Elevated downstream resistance increases PI primarily by decreasing the mean velocity, while increased upstream resistance or compliance can lower the index by decreasing the cerebral perfusion pressure (21) or by causing reflex downstream vasodilation (20). Still, if heart rate and blood pressure remain steady, increased PI implies increased downstream resistance in normal, healthy participants. Once again, we saw no changes.

In adults the reported MCA depth is 30 to 65 mm, and mean MCAv is 55 ± 12 cm/s (20). Our measurements fell within these ranges. We are therefore confident that we consistently measured velocity in the MCA, not in a different artery.

Effects of phase or epoch

Measurements varied with time during this experiment, but not with gas conditions. The effects of phase or epoch, both indicators of elapsed time, were present without interaction with the gas conditions. Blood pressure increased gradually, a probable indication of discomfort, corroborated by the increase in symptom-reporting. Participants sat still for more than 90 minutes with gear on their heads, a mask sealed to their faces, a blood pressure cuff on one arm, a finger cuff pulsating on one hand, and posture and neck position constrained by the position of the breathing block. Heart rate showed an apparent nadir at the end of Phase 2 when participants had been seated, not moving, for more than 1 hr 20 min, and before the anticipation of the end of the session that accompanied the Phase 3 visual fixation period. End-tidal CO_2 remained well-controlled and steady despite the changes in inspired gas. This was important, because MCAv is sensitive to changes in arterial CO_2 partial pressure (22).

MCAv declined from baseline to Phase 1 even when the gas was 21% O_2 throughout, and then remained fairly steady. This most likely represents some reduction in the acoustic coupling between the TCD probes and the head because of minor shifting of instrumentation early in the sessions. The straps of the breathing mask lay on top of the TCD headband, possibly transmitting any traction on the mouthpiece to the TCD probes as the participants assumed a more settled position and as the stretch in the headband itself equalized.

During the visual fixation tasks, constant f_MCAv with increasing MAP implied a decrease in vascular conductance index f_CI, calculated from MAP and MCAv. Measured PI decreased with time, suggesting that the arterial constriction responsible for the increased blood pressure was upstream of the measurement site.

Effects of epoch without interaction with gas condition were seen at the starts of Phase 2 and Phase 3. Although the participants had no tasks to perform in the first minutes of Phase 2 they were permitted to close their eyes and no longer had the visual fixation to maintain. They began Phase 3 with the second vigilance task, also a change in mental activity from the period of visual fixation immediately preceding. There were only marginal differences in TCD variables between vigilance tasks, but PI was lower during the second Sternberg test than during the first. All effects were related to the passage of time during the experiment, not to breathing gas.

Effects of gas condition, interaction of gas condition and phase

Effects of the experimental interventions would have been evident in interaction between experimental phase or epoch of a phase and gas condition. No such interactions were found despite clear changes in the inspired gas. If any changes were caused by changing P_aO_2, other mechanisms compensated to keep MAP, HR, MCAv, and PI stable. There were no changes in the results of the vigilance or memory tasks. Even with 50 minutes of 35% O_2 (Gas condition 35%O_2S) we saw no effects of PO_2 on MCAv. Possible explanations are either that 1) the middle cerebral artery, known to be sensitive to changes in P_aCO_2 (22) and to hypoxia (13, 14) is not sensitive to P_aO_2, or 2) PO_2 changes larger than those we used, (here, alveolar PO_2 measured as end-tidal gas ranged from 90 to 230 Torr), are needed to provoke alterations in the variables we measured. Both are possible: the literature suggests that the middle cerebral artery is insensitive to hyperoxia (23), and that grey matter perfusion remains at 97% control with 40% O_2 inspired at ground level (15).

Cognitive performance

In this experiment gas conditions (35% vs. 21% O_2) had no effect on the cognitive task performance. In contrast, Damato et.al (4) showed improved vigilance test performance when participants breathed 100% O_2 as compared to 21% O_2. The two conditions, that of those authors and that of these experiments, are too different for any comparison to be made. Further, the cognitive tasks were probably too easy in this study; most participants had very high scores under all conditions. When all scores are high, improvements are hard to discriminate.

The Environmental Symptoms Questionnaire was developed to measure effects of altitude exposure. Here we applied it to short-term hyperoxia. The effects of phase were most likely effects of time during the exposures, that is, the discomfort of the gear and irritation from breathing dry gas.

Experimental limitations

Despite the use of a continuous finger blood pressure monitor, only brachial cuff pressures are reported here. Finger pressures were not available during the times of interest because cuff pressures were measured during the periods selected for analysis. Further, the finger pressures were not recalibrated to brachial pressure when the measurement switched from one finger cuff to the other. Differences in readings

between finger cuffs were unexpectedly large. Because the measurement finger was changed every 15 minutes to reduce hand discomfort, repeated brachial calibrations each time were impractical and would have been intrusive.

We could not obtain good TCD signals in almost 20% of our volunteers. This problem was not unique to our laboratory; from 10% and 20% of evaluated populations are reported to have trans-temporal windows inadequate for successful insonation (20).

The hyperoxic stimulus used here was chosen to match oscillations of concern from an F-35 OBOGS set for composition control. This may not represent conditions for larger amplitude or longer period oscillations in inspired oxygen partial pressure. Under conditions of hyperoxia at sea level magnetic resonance imaging (MRI) techniques have found reductions in grey matter perfusion of 7% when 100% oxygen was inhaled, but of only 3% when 40% oxygen was inhaled (15). Further, we measured only 30-s gas periods, which did not permit complete changes in the gas composition to occur. Slower changes (longer periods) would come closer to the new concentration on each gas step. However, even a single switch to 35% O_2, then back to 21% O_2 after 50 minutes (35%O_2S) had no significant negative effects with either gas switch, whether within the first four minutes after the initial gas switch, 20- to 30 minutes later, or for the first 15 minutes after the return to air breathing. Still, we cannot rule out short-lasting effects of larger amplitude, longer period gas switching.

We may have measured only regions of circulation that are insensitive to hyperoxia, missing those that do react to it; in participants who breathed 100% O_2, other authors (23) found decreased cerebral blood volume and flow in some regions of the brain, but no change in TCD-measured MCAv or in MRI-measured perfusion of the frontal-parietal regions supplied by the MCA.

Short-term changes in OBOGS output oxygen fraction caused by loss of supply pressure or by other problems in the life support system can cause much larger dips or swings in PO_2, but not regular, repetitive fluctuations. The results of this experiment do not necessarily apply large transients in oxygen concentration. However, unless the changes in inspired O_2 are great enough and last long enough that the alveolar gas also shows large changes in O_2, no physiological effects can be anticipated.

CONCLUSIONS and RECOMMENDATIONS

The step-wise gas composition changes in this experiment were more sudden than any that could be experienced by a pilot breathing from an OBOGS. Gas composition changes in the mask are damped relative to those at the concentrator outlet by mixing in the life support system piping and hoses. Gas concentration changes in the lungs are damped relative to those in the mask by gas mixing in the lungs, as were the step changes produced here. Arterial PO_2 will change with fluctuations in inspired gas, but the changes reaching the brain will be more gradual and lower in amplitude than those in the inspired gas.

The absence of significant changes makes a study less interesting, but, in this case, very reassuring. Even though the measurements pertain only to the middle cerebral artery and the brain regions served by it, cognitive function as measured by tests of vigilance and short-term memory are not affected by these fluctuations in inhaled oxygen or mild hyperoxia, and the interventions do not provoke symptoms. MCAv is not affected by oxygen fluctuations more severe than those that might be delivered by an OBOGS in composition control mode, by constant mild hyperoxia, or by return to normoxia after even 50 minutes of hyperoxia. The concentration of oxygenated hemoglobin in the frontal regions is higher when the inhaled oxygen partial pressure is higher, but oxygen delivery to that area of the brain is not perturbed by any of elevated PO_2, 30-second fluctuations in inhaled PO_2, or the return to normoxic conditions as applied in this experiment. We must emphasize that other regions of the brain may be affected, though the literature indicates that changes like those measured here would be minimal overall.

Further study of larger fluctuations in PO_2 and of lower frequencies of fluctuation is warranted to explore the full range of possible occurrence in an aircraft. Additionally, techniques that explore other brain regions of interest should be applied.

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APPENDIX

Oxygen Fluctuations Study

Environmental Symptoms Questionnaire

Select a number (0-5) corresponding to how you currently feel. Please consider your answers carefully. Note that while some feelings may not occur at all, others may be on a scale.

	NOT AT ALL	SLIGHT	SOME-WHAT	MODER-ATE	QUITE A BIT	EXTREME
1. I FEEL LIGHTHEADED.	0	1	2	3	4	5
2. I HAVE A HEADACHE.	0	1	2	3	4	5
3. I FEEL SINUS PRESSURE.	0	1	2	3	4	5
4. I FEEL DIZZY	0	1	2	3	4	5
5. I FEEL FAINT.	0	1	2	3	4	5
6. MY VISION IS DIM	0	1	2	3	4	5
7. MY COORDINATION IS OFF	0	1	2	3	4	5
8. I AM SHORT OF BREATH	0	1	2	3	4	5
9. IT IS HARD TO BREATHE.	0	1	2	3	4	5
10. IT HURTS TO BREATHE.	0	1	2	3	4	5
11. MY HEART IS BEATING FAST.	0	1	2	3	4	5
12. MY HEART IS POUNDING.	0	1	2	3	4	5
13. I HAVE A CHEST PAIN.	0	1	2	3	4	5
14. I HAVE CHEST PRESSURE	0	1	2	3	4	5
15. MY HANDS ARE SHAKING/TREMBLING.	0	1	2	3	4	5
16. I HAVE A MUSCLE CRAMP.	0	1	2	3	4	5
17. I HAVE STOMACH CRAMPS	0	1	2	3	4	5
18. MY MUSCLES FEEL TIGHT OR STIFF.	0	1	2	3	4	5
19. I FEEL WEAK	0	1	2	3	4	5
20. MY LEGS OR FEET ACHE	0	1	2	3	4	5
21. MY HANDS/ARMS/SHOULDERS ACHE	0	1	2	3	4	5
22. MY BACK ACHES.	0	1	2	3	4	5
23. I HAD A STOMACHACHE.	0	1	2	3	4	5
24. I FEEL SICK TO MY STOMACH(NAUSEOUS).	0	1	2	3	4	5
25. I HAVE GAS PRESSURE.	0	1	2	3	4	5
26. I HAVE DIARRHEA.	0	1	2	3	4	5
27. I FEEL CONSTIPATED.	0	1	2	3	4	5
28. I HAVE TO URINATE MORE THAN USUAL	0	1	2	3	4	5
29. I HAVE TO URINATE LESS THAN USUAL	0	1	2	3	4	5

(Continues next page)

	NOT AT ALL	SLIGHT	SOME-WHAT	MODER-ATE	QUITE A BIT	EXTREME
30. I FEEL WARM	0	1	2	3	4	5
31. I FEEL FEVERISH	0	1	2	3	4	5
32. MY FEET ARE SWEATY	0	1	2	3	4	5
33. I AM SWEATING ALL OVER	0	1	2	3	4	5
34. MY HANDS ARE COLD	0	1	2	3	4	5
35. MY FEET ARE COLD	0	1	2	3	4	5
36. I FEEL CHILLY	0	1	2	3	4	5
37. I AM SHIVERING	0	1	2	3	4	5
38. PARTS OF MY BODY FEEL NUMB	0	1	2	3	4	5
39. MY SKIN IS BURNING OR ITCHY	0	1	2	3	4	5
40. MY EYES FEEL IRRITATED	0	1	2	3	4	5
41. MY VISION IS BLURRY	0	1	2	3	4	5
42. MY EARS FEEL BLOCKED UP	0	1	2	3	4	5
43. MY EARS ACHE	0	1	2	3	4	5
44. I CAN'T HEAR WELL	0	1	2	3	4	5
45. MY EARS ARE RINGING	0	1	2	3	4	5
46. MY NOSE FEELS STUFFED UP	0	1	2	3	4	5
47. I HAVE A RUNNY NOSE	0	1	2	3	4	5
48. I HAVE A NOSE BLEED	0	1	2	3	4	5
49. MY MOUTH IS DRY	0	1	2	3	4	5
50. MY THROAT IS SORE	0	1	2	3	4	5
51. I AM COUGHING	0	1	2	3	4	5
52. I HAVE NO APPETITE	0	1	2	3	4	5
53. I FEEL SICK	0	1	2	3	4	5
54. I FEEL HUNGOVER	0	1	2	3	4	5
55. I AM THIRSTY	0	1	2	3	4	5
56. I FEEL TIRED	0	1	2	3	4	5
57. I FEEL SLEEPY	0	1	2	3	4	5
58. I FEEL WIDE AWAKE (COULDN'T SLEEP)	0	1	2	3	4	5
59. MY CONCENTRATION IS OFF	0	1	2	3	4	5
60. I AM MORE FORGETFUL THAN USUAL	0	1	2	3	4	5
61. I FEEL WORRIED OR NERVOUS	0	1	2	3	4	5
62. I FEEL IRRITABLE	0	1	2	3	4	5