Cerebrovascular effects of 73% and 100% O_2 delivered in the laboratory: Steady hyperoxia or oscillations with normoxia

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STATEMENTS

Disclaimer

The views expressed in this technical report are those of the authors and do not 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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Conflict of Interest

The authors declare that they have no conflicts of interest.

Human Research/Institutional Review Board (IRB) statement

The study protocol was reviewed by the Naval Medical Research Unit Dayton Institutional Review Board.

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ABSTRACT

Fluctuations in inspired oxygen partial pressure (PO_2) are normal in current-generation tactical jets that deliver oxygen concentrations specific for cabin altitude. Onboard oxygen generating systems (OBOGS) provide oscillating concentrations within the acceptable band. In a previous study, our laboratory simulated fluctuations in oxygen partial pressure (PO_2) of the magnitude expected from an F-35 OBOGS. We measured average blood velocity in the middle cerebral artery (MCAv) and related variables (transcranial Doppler; TCD) and frontal cortex blood oxygenation (near infrared spectroscopy; NIRS) as well as a number of other variables, and found no significant effects of the PO_2 fluctuations. This IRB-approved study was an extension in a subset of participants examining effects of the maximum swings in PO_2 that can be generated without hypoxia. TCD and NIRS were measured for inspired gas switching between 100% O_2 and air with 30 s spent breathing each gas. Because naval aviators often fly at a cabin altitude of 8,000 ft while breathing the maximum oxygen fraction supplied by the OBOGS, measurements at the equivalent PO_2, 73% O_2 in our laboratory, were included. Effects of steady air and steady hyperoxia also were assessed. Values with disturbances of carbon dioxide (CO_2) balance generated by hyperventilation or breath-holding under all of the O_2 conditions were also measured. Ten participants from the first study returned for a single experimental session. No important effect of O_2 condition was seen during normocapnia. In contrast, hypocapnia (hyperventilation) dramatically reduced, and hypercapnia (breath-holding) unequivocally increased, MCAv, and the O_2 conditions modulated those effects. We conclude that square-wave fluctuations in inspired PO_2 with period 1 minute have little effect on circulation to or oxygenation of the brain. However, hyper- and hypoventilation are potent stimuli of changes in cerebral artery velocity, and the changes are functions of PO_2.

INTRODUCTION

Although air crew of most high-performance Navy jets breathe nominally 94% to 100% oxygen (O_2) at all cabin altitudes, those flying similar Air Force planes or the F-35 breathe lower O_2 fractions when the altitude permits. Their life support systems are designed to prevent hypoxia while also moderating hyperoxia. However, current on-board oxygen generating systems (OBOGS) commonly generate O_2 fractions that oscillate around a mean value when they are set to less than maximum O_2 output. Although O_2 partial pressure (PO_2), not O_2 fraction, drives physiological responses, PO_2, calculated as O_2 fraction multiplied by cabin pressure, varies with O_2 fraction when cabin pressure is constant. Whether oscillations in supplied PO_2 have physiological implications is unknown.

This laboratory recently investigated effects at ground level of square-wave changes in PO_2 similar to some F-35 OBOGS laboratory outputs (1), where regular, quasi-sinusoidal oscillations with periods from 30 to 60 seconds and peak-to-peak magnitude about 15% O_2, for example, from 60% to 45% O_2, have been recorded. Because much flight time occurs at cabin altitudes of 8,000 ft above mean sea level (MSL), partial pressures in the laboratory (900 ft MSL) were matched to that altitude. Specifically, oscillations from 35% O_2 to air on the ground were generated to be equivalent in partial pressure to oscillations from 45% to 30% O_2 at 8,000 ft MSL. (The minimum percentage of oxygen allowed by the oxygen schedule corresponds to 23%, O_2 in our laboratory; the 21% O_2 of air was considered to be a reasonable approximation). Each gas was presented for 30 seconds in a square wave of period 1 minute. No important effects of oxygen oscillations were found in either mean middle cerebral arterial blood velocity (MCAv) and its associated variables or in oxygenation of the frontal cortex as measured with near-infrared spectroscopy (NIRS) (2). This indicates either no effect of the oscillations or insensitivity of the measurements used. Accordingly, we undertook a small adjunct study to look at effects of much larger amplitude oscillations to amplify any effect.

The maximum amplitude oscillation that avoids hypoxia is that from 100% O_2 to air, which we presented as a square wave of period 1 minute. Because most Navy tactical jets use nearly 100% O_2 at all altitudes including ground operations, we also measured effects of steady 100% O_2. To match the PO_2 given by 100% O_2 at 8,000 ft MSL we used 73% O_2 in the laboratory, both as a steady inspired concentration and as the hyperoxic side of the square wave of gas composition. Thus, data were collected while participants breathed air, 100% O_2, oscillations between 100% O_2 and air, 73% O_2, oscillations between 73% O_2 and air, and air again. Continuous measurements were made of transcranial Doppler (TCD) ultrasound measurements of the MCAv, finger blood pressure, inspiratory flow, gas composition in the mask, and forehead NIRS signals. Variables were compared on a within-participant basis. The primary objectives were to determine whether brain blood flow, frontal cortex oxygenation, or any related variable changed in response to the extremes of hyperoxia that could be anticipated in

an aircraft or to oscillations between those extremes and normoxia (air in the laboratory).

Hyperoxia

Hyperoxia causes both systemic vasoconstriction (3) and pulmonary vasodilation (e.g., 4), through multiple mechanisms. One mechanism is the depletion of the primary endothelially-derived relaxing factor, nitric oxide (NO) (5) by reaction with superoxide (6); significant reduction in NO occurs between 10 and 20 minutes after the initiation of hyperbaric oxygenation (6). Hyperoxia also interferes with the vasodilation normally mediated by NO release from deoxyhemoglobin (7, 8), with more-immediate effect.

The increase in systemic vascular resistance (SVR) caused by vasoconstriction is associated with an increase in blood pressure (3), which activates the baroreflex to reduce heart rate (HR) (9). Baroreceptor gain (change in HR per change in blood pressure) increases with increasing PO_2 (10). Hyperoxia decreases HR through increased vagal activation (10, 11) even when blood pressure does not increase (10). Muscle sympathetic nerve activity is seen to be reduced (12).

Hyperoxia does not change oxygen delivery to the brain (3), or brain metabolic rate and glucose availability (13). However, the literature provides inconsistent results regarding cerebral hyperoxic vasoconstriction. Measurement techniques and hypocapnia may be confounding variables. For example, cerebral perfusion measured by radioactive xenon clearance and with end-tidal CO_2 held constant was shown to be reduced in people who breathed 1 ATA O_2 (14), and the authors of that study reported corroboration from some studies with hyperbaric O_2, but not from others with 0.8 ATA O_2. Magnetic resonance imaging (MRI) by arterial spin labelling and with CO_2 partial pressure allowed to vary showed that blood flow to grey matter decreased by 3% for 40% O_2, by 5% for 80% O_2, and by 7% for 100% O_2 inspired (15). However, phase contrast MRI techniques found no significant change in cerebral blood flow when participants breathed either 50% or 98% O_2 (16). Cerebral blood flow measured as the sum of volumetric flow in the carotid and vertebral arteries with end-tidal PO_2 of 430 or 320 Torr, corresponding to the average end-tidal PO_2 we measured with steady 73% O_2 and with inhaled gas fluctuating between 73% O_2 and air, respectively, and with end-tidal PCO_2 held at the normoxic value did not differ from that with normoxia (13). From MRI measurements that were made with contrast-enhancement (17) or pulsed arterial spin labelling (18), regionally heterogeneous cerebral vasoconstriction has been reported. Other studies have been summarized in a review (19).

Hypocapnia

Hyperventilation, which by definition leads to hypocapnia, causes increased HR, decreased mean arterial pressure (MAP), increased cardiac output, and an initial overall decrease in systemic vascular resistance (20) that is followed after about four minutes of hypocapnia by an increase in systemic vascular resistance (21). The MAP response is not linear with PCO_2; however, for moderate decreases from normal, MAP remains constant (22). Isocapnic hyperventilation increases HR and systolic blood pressure, which together represent a decrease in baroreceptor control (23).

Hypocapnia also causes rapid cerebral vasoconstriction, with MCAv decreasing about 12 seconds after the initial change in end-tidal CO_2. The mechanism is partially mediated through the sympathetic nervous system; sympathetic blockade eliminates about 26% of the response (24). Non-sympathetically mediated cerebral vasoconstriction during hypocapnia may be caused by pH stimulation of potassium channels, the inverse of the process known for hypercapnia (24). The relationship between MCAv and end-tidal CO_2 is sigmoidal, approximately linear near normal values, but asymptotic to a maximum during hypercapnia and to a minimum during hypocapnia (22).

Hypercapnia

Hypercapnia causes MAP to rise briskly as end-tidal CO_2 rises beyond a threshold; moderate increases in arterial PCO_2 do not change MAP (22). Forearm blood vessels begin to dilate from one to four minutes after the increase in end-tidal CO_2 (25). Hypercapnia dilates cerebral blood vessels through a mechanism that requires local acidosis surrounding the pial vessels (26) to activate ATP-sensitive potassium channels in the microvessels (27). NO production causes dilation in the larger vessels (28, 29). Sympathetic activation does not appear to play a role, though the lag between changes in end-tidal CO_2 and changes in MCAv, matches that for sympathetic control (24). The 12 second delay could also result from transport delay from the lungs to the cerebral arterial circulation and across the blood-brain barrier.

Effects of disturbance of CO_2 partial pressure (PCO_2) were investigated at each PO_2, using hyperventilation to lower arterial PCO_2 and breath-holding to increase it. A primary aim was to confirm that our techniques showed the known effects of PCO_2 and to compare the magnitudes of those effects to any O_2 effects that might be found. A secondary (exploratory) aim was to assess interactions of hyperoxia with disturbed CO_2 balance.

METHODS

The study protocol was assessed by the Naval Medical Research Unit – Dayton's Institutional Review Board and found to be in compliance with all applicable federal regulations. Ten participants from the previous study (2) agreed to return for a single session. We invited only those for whom location of the middle cerebral artery for insonation with good signal-to-noise ratio was dependable. Participants ranged in age from 19 to 42 (median 29) years, in height from 173 to 196 (median 176) cm, and in weight from 60 to 105 (median 84) kg. They received $125 for participating. Measurement techniques were very similar to those of the previous study to permit comparison.

Seated participants breathed from a silicone oronasal mask (Series 7450, Hans Rudolph, Shawnee KS) and non-rebreathing valve assembly (Model 2700, Hans Rudolph) with the inlet connected to a balloon block (2540 series, Hans Rudolph). The

balloon valve was computer-controlled to connect to one of air, 100% O_2, or 73% O_2, all at ambient pressure in 60 L gas-tight bags (Hans Rudolph).

MCAv was measured using TCD M-mode ultrasound (MultiDop T, Compumedics, Singen, Germany). Bilateral 2 MHz pulse wave probes incorporated into the DWL Diamon headband were placed over the trans-temporal windows. Both signals were collected from left and right sides, but only one of them, the signal with higher amplitude at the end of the session was used. (The Doppler signal was checked first for noise; a noisy signal would have been rejected in favor of a smaller-amplitude, clean signal).

Relative changes in oxygenated- and deoxygenated hemoglobin (OHb and HHb) in the frontal cortex were measured by NIRS. Data were recorded using Oxysoft software via bilateral Artinis PortaLite NIRS (Artinis Medical Systems, Elst, The Netherlands). Sensors were placed on the forehead at sites Fp1 and Fp2 according to the International 10-20 system for electroencephalogram placement (30).

Oxygen and carbon dioxide fractions in the mask were measured using a fast-response gas analyzer (GA-200, iWorx, Dover NH) using no filters, with the gas sampling pump set to 400 mL/min and with the gas sampling line extended into the stream of gas between the mask and the valves.

Continuous beat-to-beat blood pressure was measured using Finapres Nova (Finapres Medical Systems, Enschede, The Netherlands) finger cuffs on the left third and fourth fingers. The cuffs switched every 30 minutes, after which brachial pressure from a cuff on the left upper arm was used by the instrument to calibrate the height- and waveform-corrected finger pressures to the brachial values.

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 those from NIRS were sampled at 500 Hz, displayed, and stored using a PowerLab/LabChart data acquisition suite and extracted using DataPad (ADInstruments, Colorado Springs, CO). NIRS data were sampled at 10 Hz and collected on a second computer that ran Oxysoft.

The experiment consisted of six periods (Figure 1) with different inspired O_2 concentrations: air (21%O_2), steady 73% O_2 (73%O_2S), “fluctuations” consisting of 30 seconds of 73% O_2 alternating with 30 seconds of air (73%O_2F), steady 100% O_2 (100%O_2S), fluctuations between 100% O_2 and air (100%O_2F), and air after hyperoxia (21%O_2end). Each O_2 condition began with a normocapnic period of quiet breathing, 3 minutes for the initial air-breathing period, and 10 minutes for all subsequent periods. The sixth period (21% O_2end) ended after quiet breathing, while the others continued with voluntary hyperventilation for 40 seconds followed by up to 5 minutes of recovery, then up to two breath holds in 40 seconds followed by 2 minutes of recovery. Hyperventilation consisted of paced breathing at 20 breaths/min, with slightly elevated tidal volume. Breath holding began after normal exhalation. Five participants began

hyperoxia with 73%O_2S followed by 73%O_2F, and five with 100%O_2s followed by 100%O_2F. All participants breathed both hyperoxic gases, and all started and finished with air.

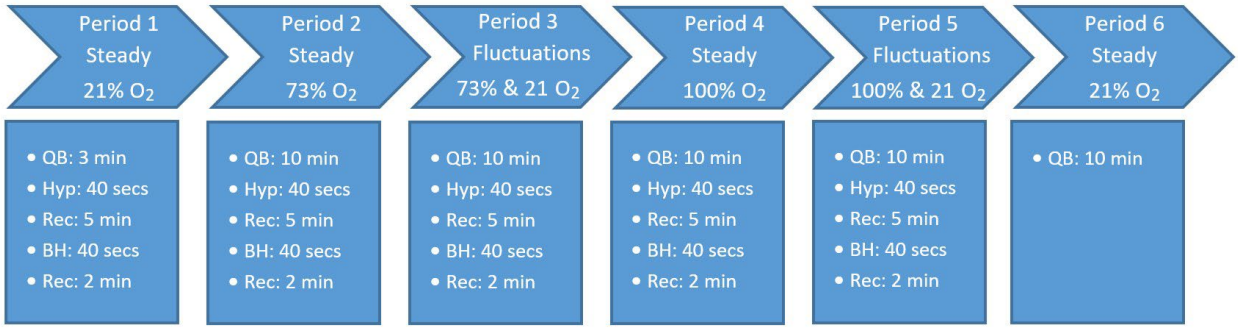

Figure 1. Timeline of data collection. “QB,” quiet breathing, “Hyp,” hyperventilation synchronized with a metronome, “Rec,” recovery time, and “BH,” breath-holding after normal exhalation. The order of 73% and 100% O_2 was counterbalanced.

Total sample size was n=10, and all were used for the TCD variables. However, two outliers with means more than 2 SD from the group mean were found in the analysis of NIRS data. Those data were removed from the analyses, resulting in n=8 for NIRS.

Statistical analyses

Gas mixing in the lungs was assessed by inspecting the raw gas concentration data. Steady state data were analyzed across one minute at the end of the normocapnic exposures (quiet breathing) and for the entire hyperventilation- or breath-hold durations. Significance was ascribed at $\alpha = 0.05$.

Cardiovascular, respiratory variables, and TCD data

MAP, mean MCAv, pulsatility index (PI), and conductivity index (cerebrovascular conductivity CVC= MCAv/MAP) were extracted beat-to-beat and averaged over the period of interest. PI is defined as the difference between maximum and minimum Doppler flow velocity divided by the mean velocity. End-tidal CO_2- and O_2 fractions were extracted breath-by-breath and averaged. These variables were assessed using two-way, repeated measures ANOVA as implemented in JMP 15 software (SAS Institute, Cary, NC) to examine the effects of O_2 fraction and the three CO_2 conditions (normocapnia, that is, spontaneous quiet breathing; hypocapnia, that is, voluntary hyperventilation; and hypercapnia, that is, during and immediately after breath holding). Separate analyses were conducted for the three conditions of steady O_2 concentration (21%O_2, 73%O_2S, and 100%O_2S) and for the two sets of data (73%O_2F and 100%O_2F) with square-wave O_2 fluctuations. To compare the effects of the fluctuations directly to air breathing an analysis that included the results for 21%O_2 with those from the fluctuating gas period was added. Tukey Honestly Significant Difference was used for post-hoc, pairwise testing.

By design, all participants in this study had completed the previous study of oxygen fluctuations with 35% O_2 as the hyperoxic gas (2), and all participants gave permission for their data to be cross-analyzed. Data from 35%O_2F were selected for comparison with the current normocapnic data. Because the angle of insonation may have differed between study days but remained constant during a session, we eliminated any question of angle by assessing MCAv and CVC as fractions of their value during air breathing at the start of each session, f_MCAv and f_CVC, respectively. One-way repeated measures ANOVA assessed the effects of O_2 fraction separately for steady and fluctuating gas conditions.

NIRS Data

Oxygenated (OHb) and deoxygenated (HHb) hemoglobin measurements were extracted and averaged according to O_2 fraction and CO_2 condition. Two-way repeated measures ANOVAs were implemented using SPSS (IBM SPSS Statistics, IBM, Armonk, NY). Analyses focused on the effects of O_2 fraction and the three CO_2 conditions, with separate analyses completed for both OHb and HHb and for steady O_2 fraction and fluctuating gas. Significant interactions were followed up with separate one-way ANOVAs. Significance was ascribed at $\alpha = 0.05$.

RESULTS

Gas mixing

Changes in inspired O_2 fraction were complete within one to two breaths in what was very close to a true step change. However, end-tidal O_2 took considerably longer (Figure 2). The initial transition from long-term air breathing to long-term hyperoxic gas breathing took at least 2 minutes to reach a new quasi-steady value (Figure 2a). Average steady-state values of end-tidal O_2 were 14% for 21%O_2, 60% for 73%O_2S, and 83% for 100%O_2S.

The final air-breathing phase, which always followed a gas fluctuation phase, showed the transition from end-tidal O_2 greater than inspired to end-tidal O_2 less than inspired (the usual situation) after one- to two minutes. End-tidal values stabilized after approximately another 45- to 60 seconds (Figure 2b).

Note that end-tidal CO_2 (the upper margin of the CO_2 tracing) showed some variation associated with the gas transitions.

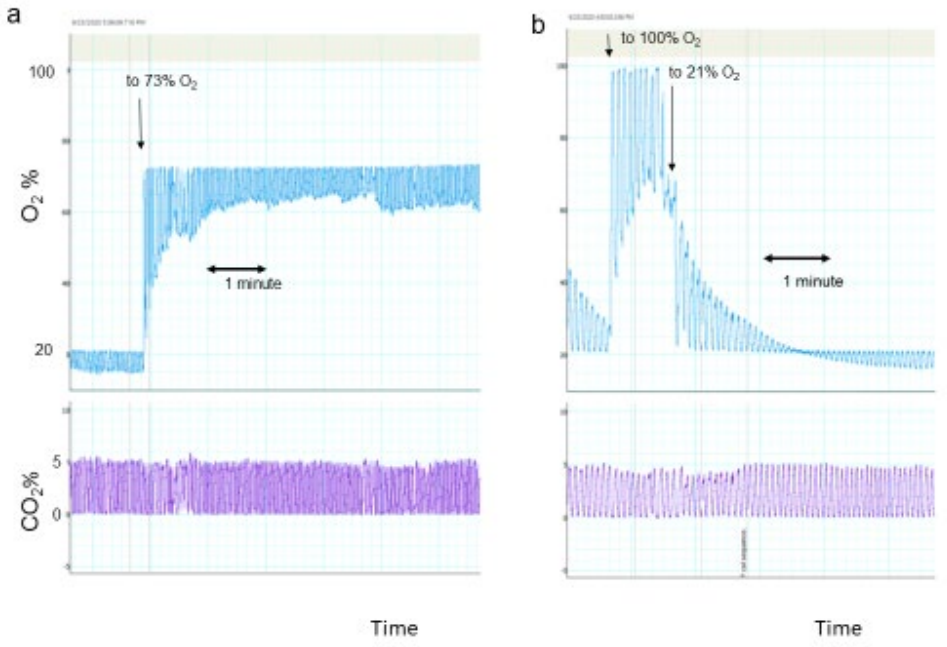

Figure 2. Gas mixing in the lungs, tracings at gas transitions during quiet breathing. a) Transition from the initial air phase (21% O_2) to steady 73% O_2. b) Transition from fluctuating 21% and 100% O_2 (100%O_2F) to the final 21% O_2 phase. Inspired gas shows as a straight line at one side of the breath-by-breath fluctuations while end-tidal gas creates the other, more irregular edge.

During the gas fluctuations with 30 seconds per inspired gas (Figure 3), on steps when 21% O_2 was inhaled, end-tidal O_2 fraction trended down with time but remained higher than inspired O_2 fraction. For steps with hyperoxic gas inhaled, end-tidal O_2 fraction trended upward with time. In neither case did the end-tidal O_2 reach a steady value within 30 seconds. Voluntary hyperventilation increased the gas mixing but did not suffice to bring end-tidal gas to a steady value in 30 seconds (Figure 3b). The average of the rippling end-tidal O_2 values was 48% for 73%O_2F and 55% for 100%O_2F.

During spontaneous breathing with oxygen fluctuations (Figure 3) end-tidal CO_2 appears to change when end-tidal O_2 changes, increasing slightly when the O_2 dips and decreasing when it climbs. With 73%O_2F, the correlation between mean end-tidal O_2 and mean end-tidal CO_2 with each gas inhaled was marginal (r = –0.25, df = 41, t = –1.98, p = 0 052). With 100%O_2F, the correlation was statistically significant (r = –0.34, t = –2.72, df = 42, p < 0.009). In neither case was the CO_2 difference physiologically important (median Δs 0.09% and 0.17% CO_2 for 73%O_2F and 100%O_2F, respectively).

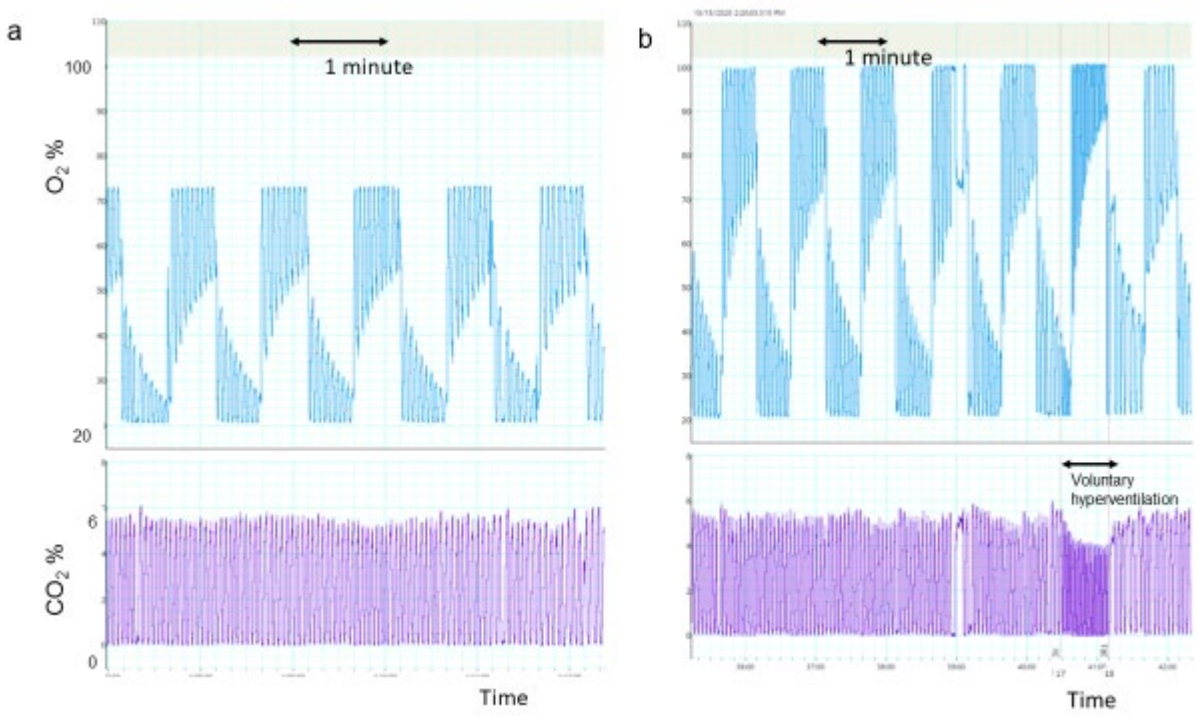

Figure 3. Gas mixing in the lungs, samples of tracings during gas fluctuations, a) 73%O_2F, quiet breathing only; b) 100%O_2F, quiet breathing and voluntary hyperventilation.

Time effects

The measurements with 21%O_2 and 21%O_2end were approximately 80 minutes apart. Paired *t*-tests (df = 9) showed no difference in MCAv (t = 0.60, p>0.56) or PI (t = 1.70, p>0.12). Although HR did not change (t = 0.52, p>0.6), MAP increased significantly (mean difference = 7.5 mmHg, t = 2.88, p<0.02) and CVC decreased (mean difference =–0.04 (cm/s)/mmHg, t = 2.36, p<0.04). End-tidal CO_2 decreased slightly, from 5.4 to 5.2% (t = 2.81, p<0.03).

Steady state measurements

Blood pressure, heart rate, end-tidal CO_2

MAP (Figure 4), for steady O_2 concentrations, was influenced by both O_2 fraction [F(2,18)=4.29, p<0.03] and by CO_2 condition [F(92,18)=22.99, p<0.0001], but at most marginally by the interaction term [F(4,36)=2.48, p=0.061]. For fluctuating O_2 conditions, MAP showed no effect of O_2 fraction [F(1,9)=1.38, p>0.2] and no interaction between O_2 fraction and CO_2 condition [F(2,18)=0.22, p>0.8], but a significant main effect of CO_2 condition [F(2,18)=12.7, p=0.0004]. The additional check of fluctuating gas conditions against 21%O_2 confirmed the lack of effect of the O_2 oscillations.

With steady O_2 conditions, MAP was lower during hypocapnia than during normocapnia (p<0.03) and lower during normocapnia than during hypercapnia (p=0.003). With

fluctuating O_2, MAP was lower during hypocapnia than during either normocapnia (p=0.039) or hypercapnia (p=0.0002), but normocapnia and hypercapnia were not significantly different.

MAP with 100%O_2S was significantly higher than with 21%O_2, but a separate analysis showed that MAP with 21%O_2end, shown for reference on Figure 4, was not different from that with 100%O_2S. MAP with 73%O_2S was intermediate, different from neither 21%O_2 nor 100%O_2S.

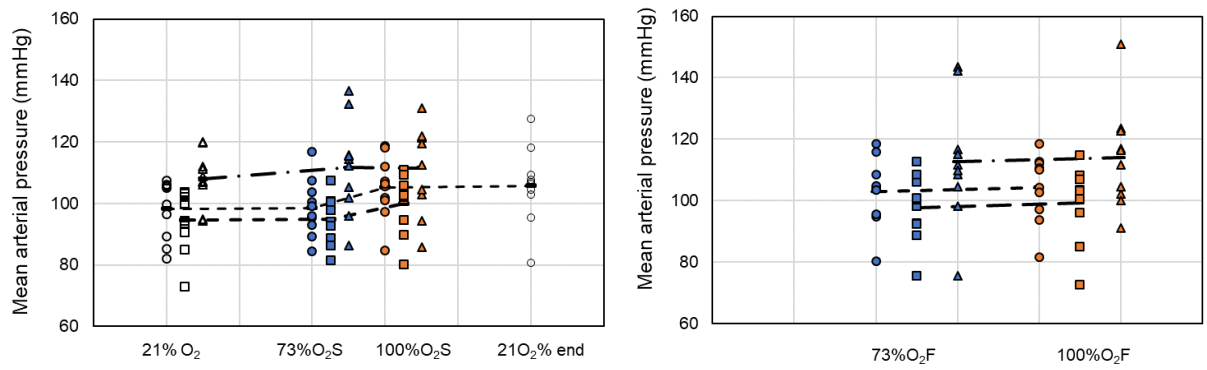

Figure 4. Mean arterial pressure as a function of O_2 fraction and CO_2 condition.

○□Δ 21% O_2 (air), ●■▲ 73% O_2, ●■▲ 100% O_2.

○●, ▬ ▬ normocapnia; □■■, ▬ ▬ hypocapnia; Δ▲▲, ▬ · ▬ hypercapnia.

Means for each O_2 fraction are connected by the lines. The O_2 fraction listed for fluctuating gas is the hyperoxic component of the oscillation.

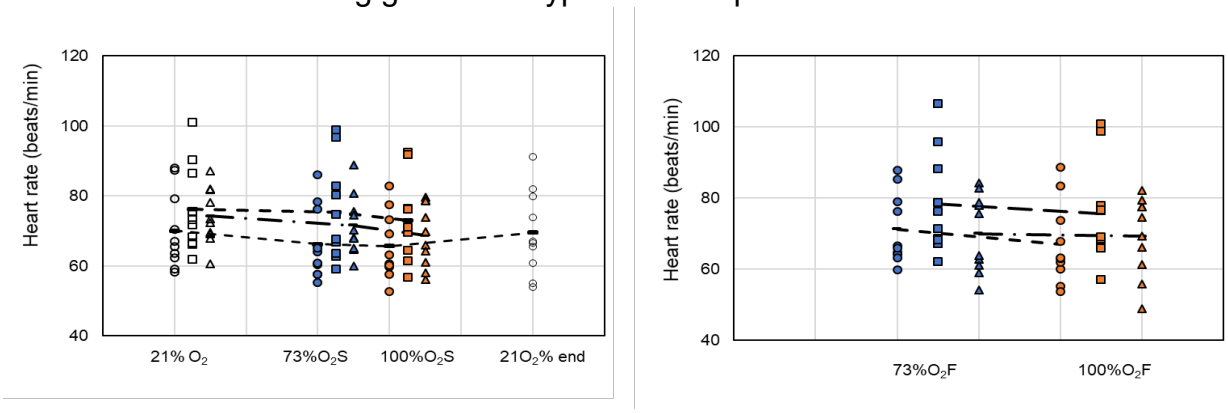

Figure 5. Heart rate as a function of O_2fraction and CO_2 condition.

○□Δ 21% O_2 (air), ●■▲ 73% O_2, ●■▲ 100% O_2.

○●, ▬ ▬ normocapnia; □■■, ▬ ▬ hypocapnia; Δ▲▲, ▬ · ▬ hypercapnia.

Means for each O_2 fraction are connected by the lines. The O_2 fraction listed for fluctuating gas is the hyperoxic component of the oscillation.

HR with steady O_2 varied with all of O_2 fraction [$F(2,18)=5.7$, $p<0.02$], CO_2 condition [$F(2,18)=6.56$, $p<0.008$], and the interaction [$F(4,36)=3.21$, $p<0.03$]. With fluctuating O_2, HR showed a main effect of O_2 fraction [$F(2,18)=5.7$, $p<0.02$], an effect of the interaction term [$F(4,36)=3.2$, $p<0.03$], and a marginal effect of CO_2 condition [$F(2,18)=3.3$, $p=0.059$] (Figure 5). In the expanded comparison including 21% O_2 with the fluctuating gases the only significant effect was that of O_2 [$F(2,18)=4.2$, $p<0.03$].

For steady O_2, HR with normocapnic 100% O_2S and 73%O_2S was lowest, not distinguishable between the two hyperoxic O_2 fractions, but significantly different from that with normocapnic 21%O_2 and that during hyperventilation. HR was highest during hyperventilation, where the values did not differ across O_2 fraction. HR during breath hold was intermediate, not different from either the lowest or highest values, yet statistically greater than that with normocapnic 21%O_2. With fluctuating O_2, HR was significantly lower during normoxic 100%O_2F than during either quiet breathing or hyperventilation with 73%O_2F or 73%O_2F (Figure 5). In the three-way analysis with fluctuating O_2 and steady air, HR with 21%O_2 did not differ from that with 73%O_2F.

End-tidal CO_2 (Figure 6) was tested only during quiet breathing, because the other CO_2 conditions represented deliberate manipulation. Steady and fluctuating O_2 were combined in a single analysis. End-tidal CO_2 varied with inspired O_2 [$F(4,36)=3.2$, $p<0.03$]. Specifically, end-tidal CO_2 was significantly lower with 100%O_2F than with 21%O_2, but all other values fell between the two and did not differ significantly from either extreme. Decreased end-tidal CO_2 is caused by increased minute ventilation if all other factors remain constant. During quiet breathing minute ventilation was higher during gas fluctuations than with steady inspired O_2.

All individuals decreased end-tidal CO_2 during hyperventilation, but the group of values for quiet breathing (nominally normocapnia) and hypocapnia overlapped. The values for hypercapnia on Figure 6, greater for every individual than the values during quiet breathing, represent only lower bounds on alveolar CO_2 at the ends of the breath holds. Since breath holding began after a normal exhalation, participants usually ended it by inhaling, meaning that the first gas sample was usually diluted by a single inhalation.

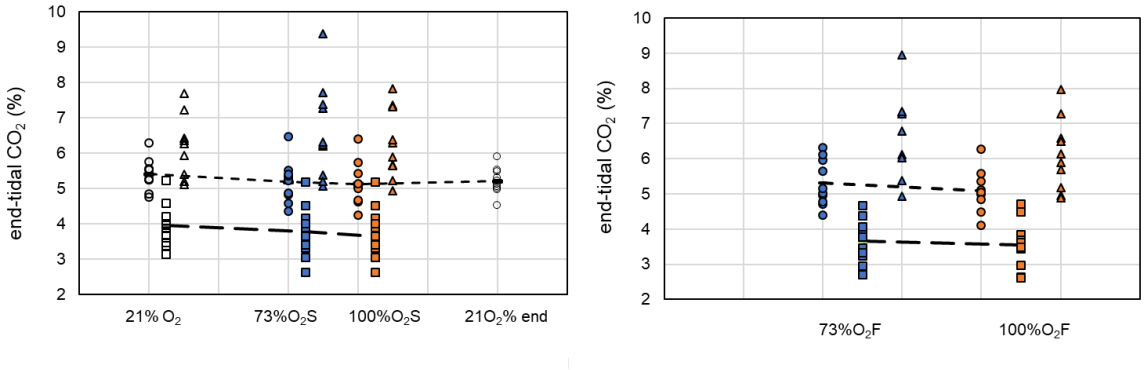

Figure 6. End-tidal CO_2 as a function of inspired O_2 fraction

○□Δ 21% O_2 (air), ●■▲ 73% O_2, ●■▲ 100% O_2.

○●●, – – normocapnia; □■■, — — hypocapnia; ΔΔ▲ hypercapnia.

Means for each O_2 fraction are connected by the lines. Hypercapnic means are not listed, because the values are lower bounds rather than measured CO_2 (see text). The O_2 fraction listed for fluctuating gas is the hyperoxic component of the oscillation.

TCD variables

Mean MCAv

MCAv (Figure 7) showed no significant main effect of O_2 fraction for steady- or fluctuating O_2 fraction [steady: $F(2,18)=2.95$, $p>0.07$; fluctuating: $F(1,9)=0.16$, $p>0.7$]. The CO_2 condition had a significant main effect for both steady-and fluctuating gas [steady: $F(2,18)=118.2$; fluctuating: $F(2,18)=61.0$, both $p<0.0001$]. The interaction of O_2 and the CO_2 condition was significant for steady gas [$F(4,36)=5.0$, $p<0.003$], but not for fluctuating gas [$F(2,18)=0.64$, $p>0.5$].

MCAv was greater during both hypercapnia and normocapnia than during hypocapnia (all $p<0.001$) overall (Table 1). For steady O_2, however, MCAv with normocapnic 21%O_2) did not differ from MCAv with hypercapnic 100%O_2S (95% confidence interval on difference –17.4 to 1.4 cm/s). Inclusion of 21%O_2 in the analysis for fluctuating gas did not introduce a significant interaction between O_2 and CO_2 [$F(4,36)=0.8$, $p>0.5$] , and MCAv with the oscillating O_2 fractions did not differ from that with steady 21%O_2 [$F(2,18)=1.3$, $p>0.27$].

Table 1. Tukey's HSD statistics for MCAv by CO_2 condition

Tukey's HSD Comparison (Ordered by differences) MCAv		Mean difference	95% Lower confidence limit	*p*
Steady O_2				
Hypercapnia	Hypocapnia	30.7 cm/s	25.6 cm/s	<0.0001
Hypercapnia	Normocapnia	15.6 cm/s	10.5 cm/s	<0.0001
Normocapnia	Hypocapnia	15.1 cm/s	10.0 cm/s	<0.0001
Fluctuating O_2				
Hypercapnia	Hypocapnia	31.4 cm/s	24.1 cm/s	<0.0001
Normocapnia	Hypocapnia	16.0 cm/s	8.7 cm/s	<0.0001
Hypercapnia	Normocapnia	15.4 cm/s	8.2 cm/s	0.0001

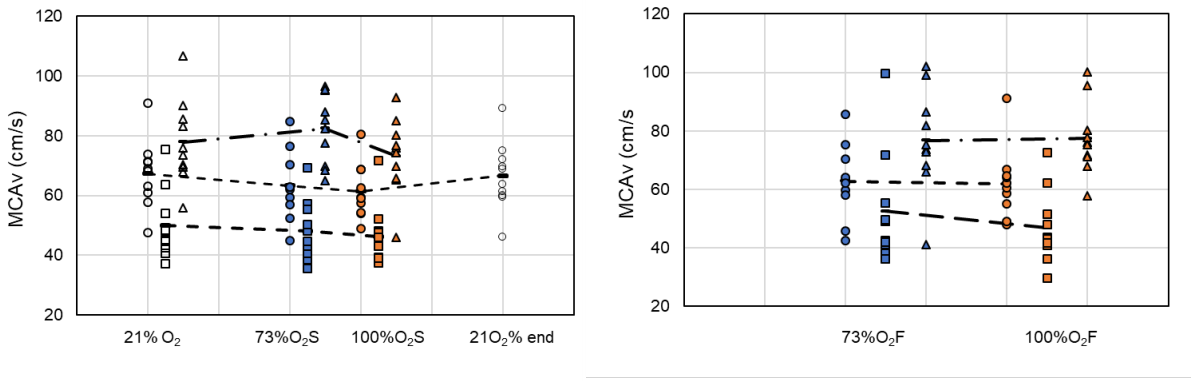

Figure 7. Mean middle cerebral artery velocity as a function of O_2 fraction and CO_2 condition.

○□Δ 21% O_2 (air), ●■▲ 73% O_2, ●■▲ 100% O_2.
○●●, – – normocapnia; □■■, — — hypocapnia; ΔΔ▲, — · — hypercapnia.

Means for each O_2 fraction are connected by the lines. The O_2 fraction listed for fluctuating gas is the hyperoxic component of the oscillation.

Gosling's pulsatility index

PI (Figure 8) also was independent of O_2 fraction [steady: $F(2,18)=0.7$, $p>0.5$; fluctuating: $F(1,9)=0.95$, $p>0.3$], and was without interaction of O_2 fraction with CO_2 condition [steady gas interaction $F(4,36)=0.15$, $p>0.9$; fluctuating gas interaction $F(2,18)=0.51$, $p>0.6$]. However, there was a main effect of CO_2 condition for both steady- and fluctuating O_2 fractions [steady: $F(2,18)=32$; fluctuating: $F(12,18) = 29.9$, both $p<0.0001$]. PI during hypocapnia was significantly greater than that during normocapnia or hypercapnia, and PI did not differ significantly between normocapnia and hypercapnia (Table 2).

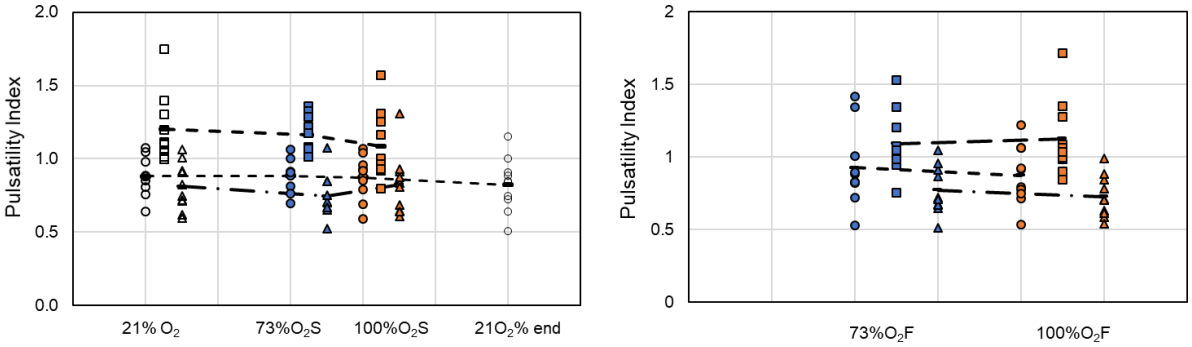

Figure 8. Pulsatility Index (PI) as a function of O_2fraction and CO_2 condition.

○□Δ 21% O_2 (air), ●■▲ 73% O_2, ●■▲ 100% O_2.

○●●, ▬ ▬normocapnia; □■■, ▬ ▬ hypocapnia; ΔΔ▲, ▬ · ▬ hypercapnia.

Means for each O_2 fraction are connected by the lines. The O_2 fraction listed for fluctuating gas is the hyperoxic component of the oscillation.

Cerebrovascular conductivity

CVC (Figure 9) differed in pattern between steady- and fluctuating O_2 fraction. For steady O_2 fractions, main effects of both gases and their interaction were significant [O_2 fraction: $F(2, 18)=8.96$, $p<0.002$; CO_2 condition: $F(2,18)=58$, $p<0.0001$; interaction: $F(4, 36)=4.4$, $p<0.006$]. However, with fluctuations in O_2 fraction without comparison to steady 21%O_2, only the main effect of CO_2 condition was significant [CO_2 condition: $F(2,18)=33.8$, $p<0.001$; O_2 fraction: $F(1,9)=0.02$, $p>0.8$; interaction: $F(2,18)=1.7$, $p>0.2$]. When CVC with 21%O_2 was compared to that with fluctuating gas, the main effect of O_2 was also significant [$F(2,18)=4.3$, $p<0.03$], still without interaction between O_2 and CO_2. CVC with 21%O_2 was marginally greater than that with 73%O_2F; the Tukey's HSD lower confidence limit on the difference was 0.0009 (cm/s)/mmHg, $p=0.0451$.

Table 2. Tukey's HSD statistics for PI by CO_2 condition

Tukey's HSD Comparison PI (Ordered by differences)		Mean difference	95% Lower confidence limit	*p*
Steady O_2				
Hypocapnia	Hypercapnia	0.39	0.26	<0.0001
Hypocapnia	Normocapnia	0.32	0.18	<0.0001
Normocapnia	Hypercapnia	0.07	-0.06	>0.05
Fluctuating O_2				
Hypocapnia	Hypercapnia	0.38	0.25	<0.0001
Hypocapnia	Normocapnia	0.28	0.15	<0.0001
Normocapnia	Hypercapnia	0.10	-0.03	>0.05

CVC during quiet breathing (normocapnia) with 100%O_2S was lower than that with 21%O_2 and higher than that for 100%O_2S and hypocapnia. CVC with 21%O_2 and quiet breathing was not different from that for hypercapnia at any O_2 fraction. With 21%O_2 CVC during hypocapnia was lower than that during normo- or hypercapnia. For all hyperoxic gas conditions, both steady and fluctuating, CVC decreased progressively from hypercapnia to normocapnia, and normocapnia to hypocapnia (Table 3).

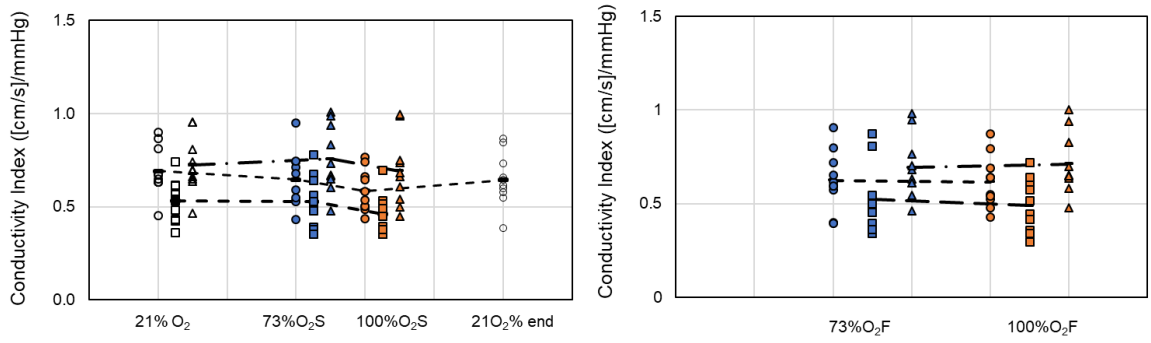

Figure 9. Cerebrovascular conductivity (CVC = MCAv/MAP) as a function of O_2 fraction and CO_2 condition.

○□Δ 21% O_2 (air), ●■▲ 73% O_2, ●■▲ 100% O_2.

○●●, – – normocapnia; □■■, — — hypocapnia; ΔΔ▲, — · — hypercapnia.

Means for each O_2 fraction are connected by the lines. The O_2 fraction listed for fluctuating gas is the hyperoxic component of the oscillation.

Table 3. Tukey's HSD comparison of O_2 – CO_2 interactions for CVC

CVC Steady O_2						
Condition, in order of decreasing CVC	Columns without the same letter differ (p>0.05; q<3.29)					Least square means
73%O_2S hypercapnic	A					0.76
21%O_2 hypercapnic	A					0.73
100%O_2S hypercapnic	A	B				0.71
21%O_2 normocapnic	A	B				0.69
73%O_2S normocapnic		B	C			0.62
100%O_2S normocapnic			C	D		0.59
21%O_2 hypocapnic			C	D		0.53
73%O_2S hypocapnic				D	E	0.51
100%O_2S hypocapnic					E	0.46

CVC Fluctuating O_2				
Tukey's HSD Comparison PI (Ordered by differences)		Mean difference	95% Lower confidence limit	p
Hypercapnia	Hypocapnia	0.22	0.15	<0.0001
Normocapnia	Hypocapnia	0.14	0.07	0.0002
Hypercapnia	Normocapnia	0.08	0.01	<0.02

Comparison with previous values for 35%O_2S and 35%O_2F

The data including 35%O_2 data are shown in Figure 10. For f_MCAv with 35%O_2 included, O_2 fraction had a possible marginal effect on MCAv with steady O_2 fraction [steady gas: F(3,27)= 2.7, p>0.06; fluctuating gas [F(2,18)=2.1, p>0.1]. PI with 35% O_2 included showed no effect of O_2 fraction [steady: F(3,27) = 0.95, p>0.4; fluctuating F(2,18)=1.5, p>0.2]. However, f_CVC showed a significant effect of O_2 fraction for steady gas [F(3.26)=5.1, p<0.007], with significantly lower f_CVC with 100%O_2S than with any of the other O_2 fractions. During gas fluctuations f_CVC was not affected by O_2 fraction [F(2,16)=0.3, p>0.7].

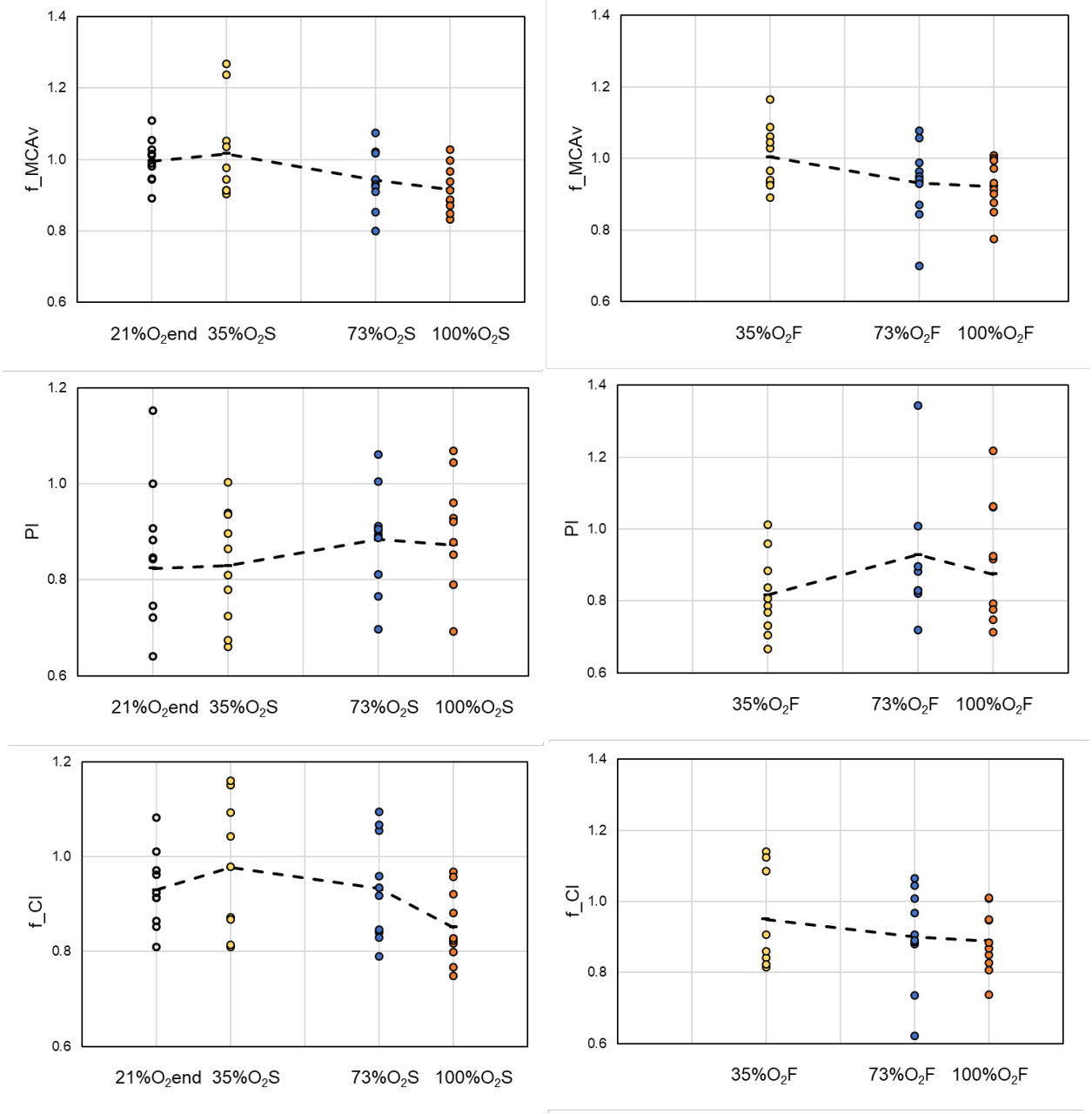

Figure 10. TCD variables during normocapnia, including 35%O_2. MCAv and CVC are expressed as fractions of air-breathing baseline. ○: 21% O_2 (air), ● 35% O_2, ●: 73% O_2, ●: 100% O_2. Normocapnia. Means for each O_2 fraction are connected with a dashed line.

Mean end-tidal CO_2 did not change with inspired oxygen fraction [steady O2: $F(3,27)=0.5$, $p>0.6$; fluctuating O_2: $F(2,16.42)=1.2$, $p>0.6$]. The variance was also not different, though the number of outliers was apparently greater with the higher levels of hyperoxia than with 21% or 35% O_2. (Figure 11).

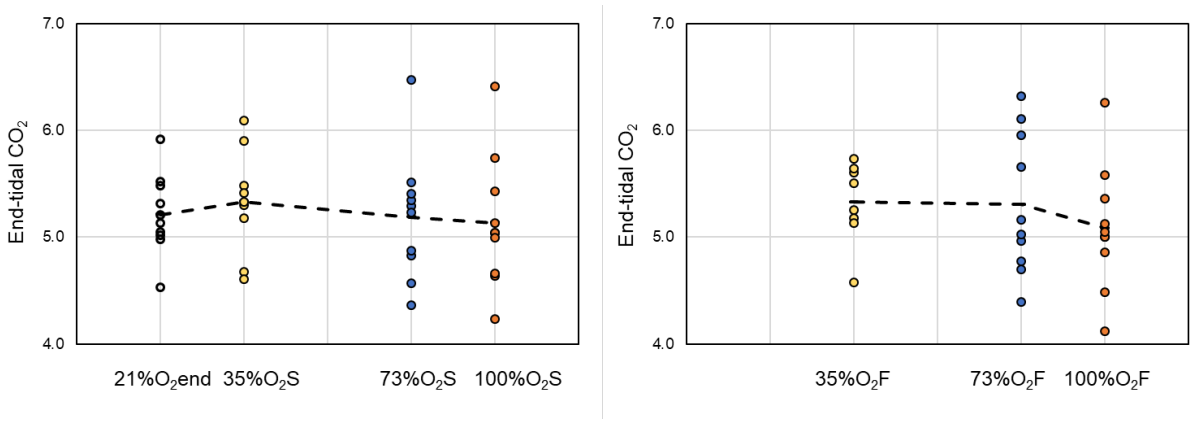

Figure 11. End-tidal CO_2 during quiet, spontaneous breathing (nominally normocapnia) as a function of inspired O_2 fraction, data for 35%O_2 included. ○: 21% O_2 (air), ●: 73% O_2, ● 35% O_2, ●: 100% O_2. Normocapnia. Means are connected with a dashed line.

NIRS

Oxygenated hemoglobin

OHb in the frontal cortex for steady O_2 fractions (Figure 12A) showed a main effect of O_2 fraction [F(2,6)=25.7, p<0.001], a main effect of CO_2 condition [F(2,6)=30.505, p<0.001], but no significant interaction between O_2 fraction and CO_2 condition. However, for fluctuating gas fractions (Figure 12B), only the main effect of CO_2 condition was significant [CO_2 condition: F(2,6)=9.052, p=0.003; O2 fraction: F(1,7)=0.52, p>0.4; interaction F(2,6)=.14, p>0.8].

OHb for 21%O_2 was significantly less than that with either 73%O_2S (p=0.002) or 100%O_2S (p=0.001), and OHb with 73%O_2S was significantly less (p=0.035) than that with 100%O_2S. OHb with hypercapnia was significantly greater than that with either normocapnia (p<0.001) or hypocapnia (p=0.001). For fluctuating gas, OHb with hypercapnia was significantly greater than that with either normocapnia (p=0.027) or hypocapnia (p=0.017), and OHb with normocapnia was significantly greater (p=0.026) than that with hypocapnia.

Deoxygenated hemoglobin

HHb in the frontal cortex for steady O_2 fractions (Figure 12C) showed a main effect of O_2 fraction [F(2,6)=71.8, p<0.001], CO_2 condition [F(2,6)=4.97, p=0.023], and an interaction [F(4,4)=5.70, p=0.002]. For fluctuating O_2 fractions (Figure 12D), HHb also showed a main effect of O_2 fraction, [F(1,7)=40.5, p<0.001], a main effect of CO_2 condition [F(2,6)=9.29, p=0.003], and a significant interaction F(2,6)=5.536, p=0.017].

For steady O_2 fraction, HHb with 21%O_2 was significantly greater than that with 73%O_2S or with 100%O_2S (both p<0.001), and HHb with 73%O_2S was significantly greater than that with 100%O_2S (p=0.01). Further analyses showed significant CO_2 effects only during 100%O_2S [F(2,6)=12.5, p=0.001], where HHb was significantly lower during hypercapnia compared to normocapnia (p=0.002) or hypocapnia (p=0.024), but there was no significant difference between normocapnia and hypocapnia (p=0.057).

For fluctuating O_2 fractions, HHb with 73%O_2F was significantly greater than that with 100%O_2F (p<0.001). For 73%O_2F, HHb during normocapnia was less than that during hypocapnia (p=0.045) but not significantly different from that during hypercapnia (p=0.058), though HHb did not differ between hypocapnia and hypercapnia (p>0.8). For 100%O_2F, HHb during hypocapnia was significantly greater than with either normocapnia (p<0.001) or hypercapnia (p=0.003), not different between normocapnia and hypercapnia (p>0.15).

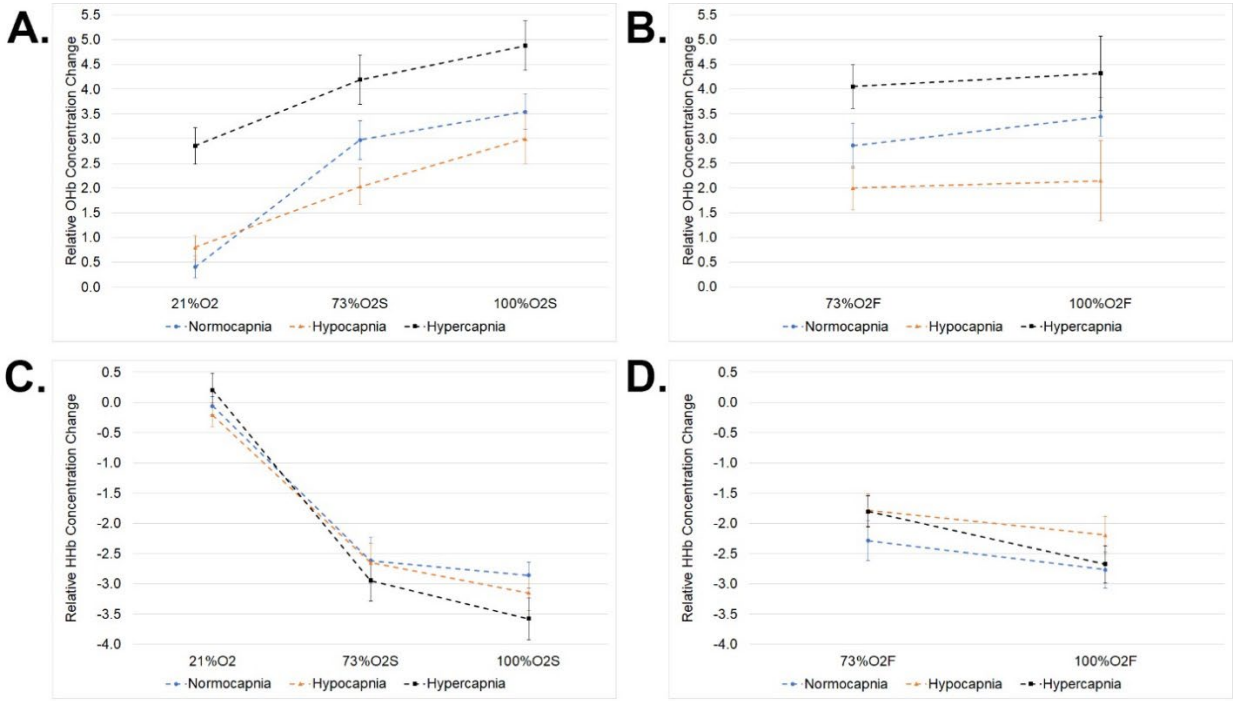

Figure 12. NIRS data as functions of inspired O_2 fraction. A) ΔOHb, steady inspired gas fraction; B) ΔOHb, fluctuating gas fractions; c) ΔHHb, steady inspired gas fraction; D) ΔHHb, fluctuating gas fractions; ●: normocapnia (spontaneous quiet breathing); ●: hypocapnia (hyperventilation); ●: hypercapnia (breath holding). Means are connected with dashed lines. Error bars represent standard error of the mean.

DISCUSSION

We previously found no changes in MCAv, PI, or CVC related to breathing of mildly hyperoxic gas (35% on the ground) or to oscillations on the ground between air and 35% O_2 (2). In this study we expanded the hyperoxic range to both the most common PO_2 for Navy aviators, equivalent to 100% O_2 at 8,000 ft MSL, and to nearly the

maximum PO_2 they experience, 100% O_2 on the ground in our laboratory, at approximately 900 ft MSL. We supplied gas oscillating between the two hyperoxic gases and air while maintaining 30-second durations of each gas. Thus, the gas fluctuations here were much greater in amplitude but similar in frequency to those seen from some OBOGS concentrators. We found few effects of the inspired O_2 fraction or the oscillations on parameters of flow in the MCA, even though 100% O_2 increased MAP and decreased HR. Only CVC, a measure that includes MAP, showed any effect of O_2 during quiet breathing; CVC with 100%O_2S was lower than the other values. Similarly, we observed increases in OHb and decreases in HHb concentrations in frontal cortex under hyperoxic conditions, but no effects specific to the oscillations emerged.

We also manipulated CO_2 homeostasis by voluntary hyperventilation or by breath-holding. Relative to that during normoxia, MCAv increased during breath holding (hypercapnia) and decreased during voluntary hyperventilation (hypocapnia); PI increased during hypocapnia; and CVC increased with hypercapnia and decreased with hypocapnia. The increase in MCAv caused by hypercapnia was eliminated by 100%O_2S but not by 73%O_2S or either of the fluctuating gases.

The major question addressed in this study was whether hyperoxia or rapid oscillations in PO_2 impairs O_2 delivery to the brain. For these experiments, our surrogates for O_2 delivery were MCAv (normal hemoglobin saturation assumed) and frontal cortex oxygenation. Neither showed evidence of impairment.

Imposed changes in O_2

Changes in inspired gas were almost instantaneous; the balloon valve was on the low pressure side of the gas delivery system, immediately upstream of the one-way, non-rebreathing valve attached to the participant's mask. Changes that reach a pilot from oscillations in high-pressure gas at an O_2 concentrator outlet are relatively delayed and averaged by mixing in the life support system before they reach the breathing regulator. Thus, our experimental conditions were the worst case for rapid changes as well as for non-hypoxic oxygen oscillations.

The measured changes in end-tidal O_2 fraction resembled the expected pattern of serial dilution of fresh gas with the gas resident in the lungs. Serial dilution with complete mixing generates an exponential curve if the breaths remain constant in size and frequency. For the gas steps of this experiment, 30 seconds was inadequate for end-tidal gas, a sample of alveolar gas, to plateau; a plateau required at least two minutes. Instead, the alveolar gas oscillated between approximately 35% and 60% O_2 (73%O_2F) or 40% and 70% O_2 (100%O_2F) in a waveform, period 1 minute, comprised of alternating increasing and decreasing exponentials towards an asymptote. The fluctuations of PO_2 in arterial blood were presumably damped further by the distribution of pulmonary blood transit times. The lungs did not eliminate the inspired oscillations, but, like any plenum or mixing chamber, they damped them.

Arterial gas partial pressures influence cardiovascular and other physiological processes. Arterial PO_2, which we did not measure directly, depends on alveolar PO_2, and end-tidal gas is a sample of alveolar gas during expiration. Alveolar PO_2 can be approximated as the product of the end-tidal dry gas O_2 fraction (measured) and the ambient barometric pressure corrected for 100% humidity within the lungs, minus the alveolar PCO_2. Barometric pressure in our laboratory is nominally 730 Torr. The vapor pressure of water in the lungs (at body temperature) is 47 Torr. Thus, with alveolar PCO_2 assumed to be 40 Torr during quiet breathing, alveolar PO_2 was approximately 570 Torr for 100%O_2S, 410 Torr for 73%O_2S, and 96 Torr for 21%O_2. During gas fluctuations, the time-averaged alveolar PO_2 for 100%O_2F was 380 Torr, oscillating between about 270 and 480 Torr and that for 73%O_2F was 320 Torr, oscillating between approximately 240 and 410 Torr.

Anatomy

The left and right internal carotid arteries and left and right vertebral arteries supply the interlinked arteries that form the Circle of Willis. From there, the cerebral arteries transport blood to the cerebral circulation, the anterior and middle cerebral arteries to the forebrain, and the posterior cerebral arteries to the occipital lobe (31). In this experiment we insonated left and right middle cerebral arteries, that is, conduits from the Circle of Willis to the forebrain.

Downstream, the middle cerebral arteries branch multiple times to supply arterioles on the brain surface. Some of these, the pial vessels, surrounded by cerebrospinal fluid, are innervated by the peripheral nervous system, and are very sensitive to neurotransmitters and CO_2 partial pressure. The pial vessels supply smaller, parenchymal arterioles that penetrate the brain structure, and the parenchymal arterioles supply the cerebral capillary system. (The membranes of the pial and parenchymal vessels and of the capillaries constitute the blood-brain barrier). Brain venous drainage is interconnected and of sufficiently-low resistance to flow that blood in the cerebral venous system is at essentially the same pressure as that of the great veins into which it drains (31, 36). Blood pressure in the central veins is approximately ambient (zero relative to the body surface).

Although blood flow in the systemic circulation is controlled almost entirely by arteriolar resistance, the control of cerebral blood flow in the face of hypoxia, hypercapnia, and hypocapnia splits about evenly between the internal carotid and cerebral arteries and the pial vessels (32). Thus, the assumption that middle cerebral artery diameter remains constant is invalid during exposure to hypoxia, hypercapnia, and hypocapnia (33, 34), and is suspect during exposure to hyperoxia.

Measurements at steady state

Mean arterial pressure

MAP drives blood flow through the entire circulatory system from the large arteries to the central veins. However, as in this study, MAP is often used as a surrogate for cerebral perfusion pressure; in normotensive individuals at rest, the arterial pressure in the middle cerebral artery is only approximately 15 mmHg lower than that in the brachial artery (35), and the pressure in the cerebral venous system approximately equals that in the central veins (31, 36).

Measurements of MAP are needed to help interpret flow and velocity variables as well as to determine the physiological state of the participant. The brain maintains very steady blood flow in the face of changes in perfusion pressure by adjusting both arteriolar and large cerebral artery diameters (32), in the phenomenon termed cerebral autoregulation. Small changes in MAP therefore are not expected to change flow, but may be associated with changes in MCAv, PI, and CVC.

We noted a small increase in MAP across the course of the experiment from 21%O_2 to 21%O_2end. Because MAP with 21%O_2end did not differ from MAP with 100%O_2S, it is tempting to suspect a lack of recovery. However, although, fluctuating gas always followed steady gas, and 21% O_2 always book-ended the data collection, the order of presentation of 73% and 100% O_2 was counterbalanced; 100%O_2S was the second condition as often as it was the fourth one. In the previous study with similar instrumentation and duration (2) we observed an increase in MAP with time during an air-breathing time-control measurement. The increase in MAP from the start to the end of the studies most likely resulted from participant discomfort, with the effects of time in the measurements with 73% and 100% O_2 obscured by the counterbalancing. The combined instrumentation – forehead NIRS sensors, TCD probes and headband, breathing mask and head harness to hold it, finger blood pressure cuffs, and accompanying constrained posture was quite burdensome.

MAP increased from baseline (21%O_2) with 100%O_2S, but only by an average of 0.9 Torr. It did not change statistically with 73%O_2S or with either fluctuating O_2 condition. It appears that arterial PO_2 calculated as 570 Torr (100%O_2S) was sufficient to cause a consistent, small systemic vasoconstriction, but PO_2 of 410 Torr (73%O_2S) or lower was not. MAP also increased with hypercapnia and decreased with hypocapnia, as the literature leads us to expect (20, 22, 25). None of the changes in MAP was physiologically important; changes were similar in magnitude to the time effect that we presume was caused by cumulative discomfort.

Heart rate

HR during quiet breathing (putative normocapnia) was lower than that with 21%O_2 with 73%O_2S, 100%O_2S, and 100%O_2F, but not with 73%O_2F. The differential effects of hyperoxia on HR and MAP indicate that the changes in HR are not entirely caused by the baroreflex. Perhaps there are direct O_2 effects on the vagal activation or sympathetic withdrawal. Additionally, HR increased with hyperventilation, perhaps as a

function of greater pressure swings in the chest (23) as well as of decreased MAP.

End-tidal CO_2

End-tidal CO_2 did not change significantly with oxygen fraction during this experiment except for with 100%O_2F where the ripple in end-tidal O_2 was matched by a much smaller inverse ripple in end-tidal CO_2. End-tidal CO_2 during spontaneous breathing has been shown by others to decrease with hyperoxia (37). The small sample size may have obscured changes.

Mean middle cerebral arterial flow velocity

We measured mean flow velocity, the average across the arterial cross-section averaged across a number of cardiac cycles. Peak velocity, which we also measured, is much more difficult to interpret. Pulsatile flow in arteries is fully-describable only through complicated equations with time-, radial- and axial terms. The Hagen-Poiseuille equation, though cited often in regard to blood flow, applies only to steady flow in a long, straight, non-elastic tube.

The variable that affects the perfusion of the brain is volumetric flow, the product of (measured) mean velocity and (unknown) arterial cross sectional area. Interpretation of changes in MCAv as changes in flow is complicated by the fact that cerebral blood flow is regulated by changes in cross-sectional area of the larger cerebral vessels as well as by down-stream arteriolar constriction or dilation. Further, the distribution of flow differs between white- and grey matter and among regions of the brain (17, 18).

If blood flow is constant as is expected for cerebral flow with steady blood gases and functioning autoregulation, any increase in mean velocity indicates constriction of the blood vessel. If, instead, artery cross-sectional area remains constant, an assumption valid where flow is controlled entirely by arterioles, an increase in mean velocity indicates an increase in volumetric flow. However, when arterial blood gases are manipulated, neither cerebral flow nor cerebral vascular diameter can be assumed constant; hypercapnia (38), hypoxia (34, 39), hypocapnia (38), and hyperoxia (14) alter the flow maintained by cerebral autoregulation, in part through changes in the diameter of the middle cerebral artery (34). In fact, simply because mean flow in the artery is the product of mean velocity and cross-sectional area, mean velocity can remain constant if both flow and vessel diameter change and the change in flow is equal to the velocity multiplied by the change in cross-sectional area.

Other investigators have measured cerebral flow and MCAv simultaneously. In one study where participants breathed 100% O_2 at sea level while CO_2 was manipulated, fractional changes in MCAv underestimated fractional changes in cerebral blood flow during hypercapnia and overestimated them during hypocapnia (40). In another study, two groups of participants breathed 21% or 100% O_2 with end-tidal CO_2 held constant, while either brain blood flow (by MRI) or MCAv was measured. There were no

differences in MCAv, but a significant decrease in brain blood flow with the hyperoxia (17). There may be some confounders in that study; HR decreased with 100% O_2 only in the MRI group, who were measured on two days for the two conditions. Further, it is not clear that both groups were supine, and the duration of the measurements may have differed. Both body position and long duration might have led to atelectasis when the breathing gas was 100% O_2, in which case end-tidal PCO_2 of 40 Torr would represent hypercapnia. Further, end-tidal PCO_2 is lower in people when they are seated than when they are supine (41).

In this experiment, hyperoxia, whether steady or fluctuating, had no statistically significant effect on MCAv. Note, though, that when the data for 35%O_2 were included, MCAv during quiet breathing trended lower with 73% or 100% O_2 than with 21% or 35% O_2. However, as discussed above, the lack of change in MCAv cannot be used to say that cerebral perfusion, or even middle cerebral artery flow, does not change when inspired O_2 changes.

1. Flow may have changed, but its decrease may have been approximately equal to the unchanged MCAv multiplied by the change in cross-sectional area at the measurement site, as described above.

2. MCAv may have remained constant while cerebral flow redistributed because of regionally heterogeneous vasoconstriction that has been reported during hyperoxia (17, 18);

3. The durations of hyperoxic exposure chosen in this study, chosen based on dynamics reported elsewhere (15), may have been too short for development of vasoconstriction. Other investigators (6) found significant reduction in NO after 20- but not after 10 minutes of hyperoxia.

4. Changes in MCAv with hypercapnia and 100% O_2 were shown to underestimate changes in cerebral blood flow (40), yet we know nothing of the relationship between MCAv and cerebral blood flow with other O_2 fractions.

In contrast, changes in CO_2 clearly altered MCAv. The average decrease of end-tidal CO_2 from 5.2 to 4.0% was associated with decreases in MCAv from 62 to 47 cm/s, while breath-holding drove MCAv to an average of 77 cm/s. These blood velocity changes began nearly instantaneously with the altered CO_2.

Gosling's pulsatility index

PI, the ratio of the peak-to-peak amplitude of the velocity waveform to the mean velocity, is most commonly considered to be an indicator of vascular resistance downstream of the MCA, that is, of distal cerebrovascular resistance. PI increases also with increasing compliance (decreasing cerebral blood volume) and HR, and is negatively correlated with cerebral perfusion pressure (42). PI has normal values between 0.5 and 1.2 (43); the values we measured during quiet breathing nearly spanned that range.

Both hypocapnia and hypercapnia increased HR in our measurements, but only hypocapnia showed increased PI relative to normocapnia. In these experiments, hyperventilation lowered MAP, thus cerebral perfusion pressure, but only by 3 to 5%. Thus, the increase in PI during hypocapnia situates some vasoconstriction downstream of the middle cerebral artery, presumably in the pial vessels; the literature suggests that vasoconstriction would have occurred also in the large cerebral arteries (32). We cannot use PI to locate the vasodilation caused by hypercapnia.

Cerebrovascular conductance

Vascular conductance, the inverse of vascular resistance, is the ratio of volumetric flow to the pressure difference that drives the flow. The usual index of cerebrovascular vascular conductance from transcranial Doppler work, that was used here, CVC = MCAv / MAP (44), substitutes flow velocity for volumetric flow and MAP for cerebral perfusion pressure. For the reasons discussed above, MCAv is only a partially-valid stand-in for flow. Further, vasoconstriction between the central arteries and the middle cerebral artery itself will influence CVC.

With all caveats in mind, the influence of hyper-and hypocapnia on CVC during hyperoxia was in the direction anticipated for the vasoconstriction or vasodilation, respectively. The decrease in CVC from 21%O_2S to 100%O_2S may indicate slight vasoconstriction caused by hyperoxia.

NIRS data

The NIRS data represent changes from baseline of absorbance of the wavelengths characteristic of oxygenated and deoxygenated hemoglobin. The absorbance measures amount, not concentration, of the chromophore in a volumetric sample that includes the arterial and venous sides of the circulation. Thus, a change in total hemoglobin amount = OHB + HHb indicates a change in perfusion (blood flow per unit volume between the optodes), while an increase in OHb is an index of surplus oxygen available in the tissue relative to that at baseline.

During normocapnia (quiet breathing), steady hyperoxia led to an increase in OHb and a decrease in HHb in the frontal cortex, as expected. During gas fluctuations, the OHb and HHb values reflected approximately the averaged alveolar PO_2. Further, the change in total hemoglobin remained approximately constant with increasing O_2 fraction, steady or fluctuating, suggesting that hyperoxia did not alter frontal cortex perfusion.

Hypercapnia boosted the increase in OHb relative to the values for normocapnia for all oxygen fractions while decreasing HHb but increasing total hemoglobin. This indicates both vasodilation and an increase in the available oxygen surplus.

Hypocapnia caused no changes from normocapnia with 21% O_2, but during hyperoxia

OHb was lower, HHb higher, and total hemoglobin numerically lower than the values for normocapnia with the same oxygen fraction. Under hyperoxic conditions only, hypocapnia caused a decrease in perfusion in the frontal cortex, but that decrease still provided a surplus of OHb relative to normal (normoxic and normocapnic) conditions.

Comparison with the previous study

Data including the 35% O_2 marginal hyperoxia suggest a subtle decrease, statistically marginal in this small data set, in MCAv once alveolar PO_2 is sufficiently high. Comparison across the two studies was possible because, like this study, the 35%O_2F condition started with air breathing ("baseline"), followed by a period (20 minutes) with steady hyperoxic gas (35% O_2), which itself was followed by another period (30 minutes) with hyperoxic gas switching with air every 30 seconds. However, several details of the studies, specifically, durations of the exposures and measurement of blood pressure, differed. The gas conditions in the previous study were longer than in this study, and those in this study were thought long enough for steady states to be established. Blood pressure in the previous study was measured as brachial pressure during the periods of interest, while this study extracted MAP for the relevant time periods from continuous finger blood pressure calibrated against brachial pressure. (For two participants blood pressure was not available for the 35% O_2 measurements.) Different air data were compared to hyperoxia including 35% than were used in all the other assessments; 21%O_2 data were used as the baseline, and 21%O_2end to represent air breathing. MCAv did not differ between 21%O_2 and 21%O_2end although MAP did. Thus, the MCAv results are unlikely to have been affected by the choice of data near the end of the measurement.

Oxygen fluctuations

The measurements made during the fluctuating oxygen conditions have been discussed in conjunction with the steady values. Because nothing remarkable happened it may be easy to overlook them. Fluctuations had no major effects on any of the variables measured beyond the changes caused by the average O_2 fractions, a decreased end-tidal CO_2 during quiet breathing with 100%O_2F, and a small ripple in end-tidal CO_2 in opposite phase to the ripple in end-tidal O_2.

Summary

Spontaneous breathing (normocapnia)

During spontaneous breathing, hyperoxia had little effect. MAP increased and HR fell because of systemic effects. MCAv may have decreased slightly for both 73%O_2S and 100%O_2S, but the lack of change in PI suggests that any velocity decrease was caused by either constriction only in the vessels upstream of the middle cerebral artery or by a

matched decrease in diameter in cerebral arteries and pial vessels to keep the pulsatile and mean changes in proportion to one another. CVC decreased only with 100%O_2S, when the increased MAP dominated the calculation. Unchanged total frontal cortex hemoglobin with hyperoxia shows unaltered frontal cortex perfusion; hyperoxia increased OHb and decreased HHb complementarily.

Hyperventilation (hypocapnia)

Voluntary hyperventilation caused a decrease in MAP and an increase in HR that was not affected by the O_2 fraction and may have been a physical effect of the increased effort to breathe. MCAv decreased and PI increased, suggestive of downstream constriction. The decrease in MCAv, at least during 100%O_2S, is expected to have overestimated the decreased flow (40).

With hyperventilation during normoxia (21%O_2), neither OHb nor total frontal hemoglobin differed from normocapnic values, indicating that normal perfusion was maintained in the face of the constriction that lowered MCAv and increased PI. With hyperventilation during hyperoxia, OHb was lower than that with equivalent normocapnic conditions, but still higher than that for normoxia. HHb for the most part did not differ from that during normocapnia at the same oxygen fraction but was always lower than that during normoxia, while total frontal cortex hemoglobin, the indicator of perfusion, was numerically lower than that with normocapnia.

Hypercapnia

Hypercapnia, produced here by breath holding, caused an increase in MAP and little change in HR as averaged over the breath hold period. However, the systemic effects may have been influenced by breath holding *per se*, not only by the hypercapnia it induced. During normoxic conditions (21%O_2) MCAv increased while PI was not affected, OHb increased, HHb decreased somewhat, and total hemoglobin increased. Hypercapnia will have dilated the pial vessels (26) and the MCAv (33) to permit increased flow, an increase evident from the increase in frontal cortex perfusion. A uniform decrease in resistance in the artery and in the arterioles could explain the lack of change in pulsatility.

Hyperoxia moderated the vasodilation caused by hypercapnia, possibly by antagonizing the NO-mediated component of hypercapnic dilation (28, 29). MCAv with hypercapnia and 100%O_2S was not different from that during normocapnia and normoxia. Similarly, perfusion of the frontal cortex, represented by total frontal cortex hemoglobin, was close to that with 21%O_2 and quiet breathing, though OHb indicated a continued surplus of oxygen in the frontal cortical tissue.

Postulated protection of brain O_2 supply

Two homeostatic mechanisms may limit possible decreases in cerebral perfusion, one

based on CO_2 washout, and the other on concentrations of deoxyhemoglobin. This is literature-based conjecture that suggests that the cerebral circulation is protected against reductions in perfusion that would increase local CO_2 partial pressure or HHb much above normal.

CO2 washout If metabolically-produced CO_2 in any region of the brain is not cleared, local acidosis will cause an increase in perfusion, a regional effect, as well as an increase in minute ventilation, a systemic one.

The mild hyperventilation often reported during hyperoxia is usually considered to be a secondary cause of vasoconstriction that superimposes on that from hyperoxia. However, it is plausibly the end result of hyperoxic vasoconstriction instead. If perfusion is reduced by long-duration hyperoxia through mechanisms related to NO, CO_2 produced by brain metabolism will be cleared incompletely. Local acidosis will correct this by dilating the pial vessels. However, the acidosis will also activate respiratory chemoreceptors on the surface of the brain. Increased alveolar ventilation will reduce arterial PCO_2, and the diffusive transfer of CO_2 from CSF into the cerebral circulation will increase, helping to correct the acidosis (38). With sufficiently elevated arterial PO_2, peripheral chemoreceptors will not adjust for the arterial hypocapnia. The steady-state result will be arterial PCO_2 lower than baseline, normal cerebral PCO_2, and cerebral vasoconstriction greater that that if the alveolar PCO_2 were clamped at baseline to prevent the ventilatory clearance of acidosis.

HHB If the concentration of deoxygenated hemoglobin in any part of the circulation increases above normal while arterial hemoglobin is adequately saturated, the NO transport function of hemoglobin will dilate the microcirculation (7, 8), increasing regional perfusion to facilitate local oxygen delivery. Whatever the mechanism, it did not prevent the changes in MCAv or PI, suggesting that it acted downstream of the normal resistance vessels.

CONCLUSIONS and RECOMMENDATIONS

1. Hyperoxia during normocapnia had no effect other than to provide surplus O_2 to tissue. During hypercapnia it moderated vasodilation, while during hypocapnia it had no measurable effect.

2. For the measurements here, despite the ripple on alveolar, and thus arterial, PO_2, and mild disturbance of end-tidal CO_2, fluctuating O_2 fractions acted primarily like their averaged O_2 fraction.

3. Hypocapnia constricted blood vessels in the brain, but it did not affect frontal cortex perfusion during normoxia. Hypocapnia during hyperoxia reduced perfusion of the frontal cortex relative to that with hyperoxia alone, but relative to normoxic conditions a surplus of O_2 was delivered to tissue. For the level of hypocapnia used in these experiments the body prevented decreases in frontal cortex perfusion below normal, that is, below that with normoxic normocapnia.

4. Hypercapnia dilated blood vessels in the brain, increasing the delivered O_2 surplus. Hyperoxia moderated that effect.

5. Two physiologic mechanisms that may protect brain perfusion were postulated:

    a. The NO-cycle of hemoglobin (7, 8), that known to maintain tissue PO_2 constant in the face of varying O_2 demand, a possible explanation for the observed absence of hypocapnic constriction during normoxia;

    b. Feed-back control of tissue CO_2, where local CSF acidosis is known to cause vasodilation.

6. Gas concentration changes in the lungs were damped relative to those in inspired gas by serial dilution with lung gas from previous breaths.

7. MCAv provides a poor representation of cerebral flow. Frontal NIRS may be more representative of perfusion changes averaged over a large volume. Future studies should focus on NIRS or other measures of brain blood flow and not on velocity measurements.

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