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This project investigated the effects of elevated oxygen partial pressure, inspired CO_2, immersion, and work on ventilatory response to CO_2, brain cortical oxygenation, and the occurrence of symptoms of CNS oxygen toxicity in humans. Relative changes in cerebral oxygenation from a baseline were measured by near-infrared (NIR) spectroscopy. Ventilatory response was measured during CO_2 rebreathing in a computer-controlled, closed-circuit breathing apparatus. The results of this study suggest that inspired CO_2 is a potent instigator of CNS oxygen toxicity, more potent than oxygen alone or in combination with immersion and work. Most (31) symptoms were reported while the inspired CO_2 was 7.0% - 7.5% and PI_{O2} was 2.80 ATA. Hyperoxia, PI_{O2} = 1.75 or 2.80 ATA, leads to increased cerebrovascular oxygenation despite vasoconstriction. Inspired CO_2 reversed vasoconstriction and increasing cerebral oxygenation further. Subjects with symptoms consistent with oxygen toxicity did not show a CO_2-induced vasoconstrictive reversal despite increased cerebral oxygenation. Ventilatory response to CO_2 did not correlate well with symptom incidence but the subject who convulsed had the lowest ventilatory response to CO_2 at 0.21 ATA PI_{O2}. Facial immersion led to increased cerebrovascular blood volume and cytochrome a,a_3 oxidation at both 0.21 and 2.80 ATA PI_{O2} and with both positive and negative static lung loads.

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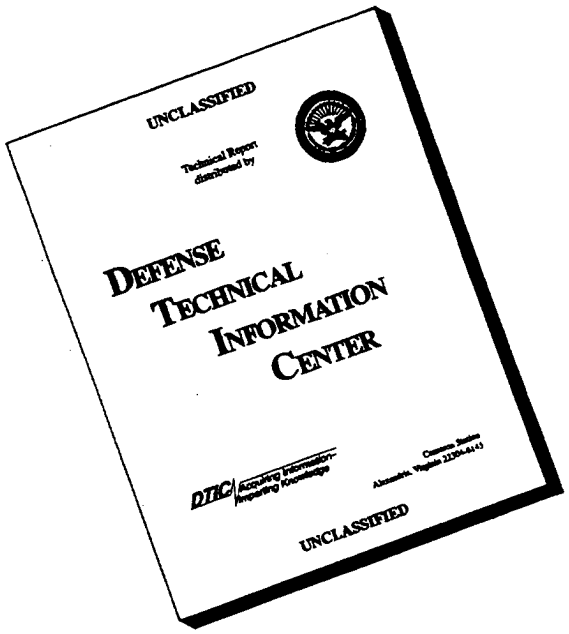

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Hyperbaric Center

January 19, 1996

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Dear Sirs:

Please find two copies of the Final Technical Report for ONR contract N00014-88-C-0400, "Factors Affecting CNS Oxygen Toxicity in Humans". Please notify me if additional copies are needed or if I may be of any assistance (919-684-6726).

Sincerely,

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Anesthesiology med CV

Factors Affecting CNS Oxygen Toxicity in Humans

Final Technical Report

January 16, 1996

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Prepared under Contract Number N00014-88-0400

for the Office of Naval Research

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ABSTRACT

The onset time of central nervous system (CNS) oxygen toxicity symptoms is reduced by elevated oxygen partial pressure, inspired CO_2, immersion, and work. This project investigated the effects of these factors in humans on ventilatory response to CO_2, brain cortical oxygenation, and the occurrence of symptoms of CNS oxygen toxicity. Relative changes in cerebral oxygenation from a baseline were measured by near-infrared (NIR) spectroscopy. Ventilatory response was measured during CO_2 rebreathing in a computer-controlled, closed-circuit breathing apparatus.

The results of this study suggest that inspired CO_2 is a potent instigator of CNS oxygen toxicity, more potent than oxygen alone or in combination with immersion and work. Thirty-three of the 35 CNS oxygen toxicity symptoms reported, including a convulsion, involved breathing inspired CO_2 at a P_{IO_2} of 2.80 ATA. Most (31) symptoms were reported while the inspired CO_2 was 7.0% - 7.5%. Hyperoxia, P_{IO_2} = 1.75 or 2.80 ATA, leads to increased cerebrovascular oxygenation despite vasoconstriction. Inspired CO_2 reversed vasoconstriction and increasing cerebral oxygenation further. Subjects with symptoms consistent with oxygen toxicity did not show a CO_2-induced vasoconstrictive reversal despite increased cerebral oxygenation. This result is contrary to previous findings (Lambertsen 1955) where inspired CO_2 caused symptoms of CNS oxygen toxicity while increasing cerebral blood flow and increasing cerebral oxygenation. This disparity is most likely due to the difference in the cerebral oxygenation measurements made. NIRS measures regional physiology while cerebral blood flow and blood sampling measurements are global physiological measurements.

Ventilatory response to CO_2 did not correlate well with symptom incidence but the subject who convulsed had the lowest ventilatory response to CO_2 with a P_{IO_2} of 0.21 ATA. The subject who convulsed also had the largest increase in cytochrome a,a_3 oxidation with

inspired CO_2. The combination of the low ventilatory response to CO_2 with increased cerebral oxygenation resulting in an oxygen seizure supports the hypothesis that susceptibility to CNS oxygen toxicity may be related to CO_2 retention as a result of depressed ventilatory response to CO_2 (Lanphier 1975).

Facial immersion lead to increased cerebrovascular blood volume and cytochrome a,a_3 oxidation at both 0.21 and 2.80 ATA P_{IO_2} and with both positive and negative static lung loads.

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ata	atmospheres absolute
BTPS	body temperature pressure saturated
CNS	central nervous system
CYT	cytochrome a,a_3
fsw	feet of sea water
Hb	deoxygenated hemoglobin
HbO_2	oxygenated hemoglobin
lpm	liters per minute
NIRS	near infrared spectroscopy
Pa_{CO_2}	arterial oxygen pressure
P_{CO_2}	carbon dioxide partial pressure
PET_{CO_2}	end-tidal carbon dioxide partial pressure
PI_{CO_2}	inspired carbon dioxide partial pressure
PI_{O_2}	inspired oxygen partial pressure
P_{O_2}	oxygen partial pressure
Pt_{CO_2}	tissue carbon dioxide partial pressure
Pt_{O_2}	tissue oxygen partial pressure
Pv_{CO_2}	venous oxygen pressure
RMV	respiratory minute volume
Sa_{O_2}	arterial oxygen saturation
STPD	standard temperature pressure dry
V_T	tidal volume

1. INTRODUCTION

1.1 Hypotheses and Objectives

During exposure to elevated O_2 partial pressure we hypothesize that inspired CO_2, immersion, and work increase oxygen delivery to the brain and raise the probability of CNS oxygen toxicity. We tested this hypothesis by correlating changes in cerebral oxygenation, ventilation, and other physiological responses with the occurrence of oxygen toxicity symptoms.

1.2 Background

Exposure to sufficient amounts of oxygen eventually becomes toxic to cells in all tissues of the body (Clark 1982). In fact, normal oxygen pressure would be toxic to most living creatures in the absence of intracellular antioxidant defenses (Fridovich 1988). The central nervous system (CNS) is acutely susceptible to the toxic effects of high partial pressures of oxygen. Regulatory mechanisms of circulation and a high metabolic rate require hyperbaric oxygen (HBO) pressures ($\geq$ 1.5 ATA O_2) to precipitate CNS oxygen toxicity in man (Lambertsen 1978). At hyperbaric oxygen pressures, oxygen delivery to the brain can exceed tolerable levels and produce signs and symptoms of oxygen toxicity. Given sufficient oxygen pressure, the onset time to the occurrence of symptoms of CNS oxygen toxicity is decreased by inspired carbon dioxide, immersion, and work (Young 1971). Any factor which enhances cerebral oxygenation has the potential to increase the rate at which CNS oxygen toxicity develops. There is a large variability in the susceptibility to CNS oxygen toxicity between subjects and in a single subject over time (Donald 1947). The use of hyperbaric oxygen for an increasing number of therapeutic purposes and greater demand for the use of

hyperoxic gas mixtures to decrease decompression time in diving, will expose many individuals to oxygen pressures capable of inducing CNS oxygen toxicity. Presently, there is no objective method to monitor the development of CNS oxygen toxicity (Fife 1991a).

An understanding of the underlying physiology of CNS oxygen toxicity is essential to developing practical strategies for avoiding it. Our approach is to measure key physiological parameters (ventilatory response to carbon dioxide, cerebral oxygenation and metabolism) under environmental perturbations which marginally increase the risk of oxygen toxicity. Our studies evaluated the effects of inspired oxygen partial pressure, oxygen exposure duration, inspired CO_2, immersion, and work on ventilatory response, cerebral oxygenation, and the incidence of CNS oxygen toxicity symptoms.

We used Near Infrared Spectroscopy (NIRS) to monitor changes in cerebral oxygenation (Appendix G). This non-invasive technique for continuous measurement of deoxygenated hemoglobin (Hb), oxygenated hemoglobin (HbO_2), blood volume and cytochrome a,a_3 oxidation-reduction (redox) level is used to assess qualitative trends in regional oxygenation and metabolism (Piantadosi 1989b). NIRS was used to track relative changes in the amount of cerebral oxygen delivery at three oxygen partial pressures (0.21, 1.75, and 2.80 ATA $P_{I_{O_2}}$) and increasing carbon dioxide to 60 torr $P_{ET_{CO_2}}$.

Ventilatory response to carbon dioxide is a traditional method for the study of respiratory function. CO_2 is the most commonly used chemical stimulus and ventilation the most commonly measured response for investigating respiratory control (Rebuck and Slusky 1981). Increased inspired oxygen pressures lead to decreased ventilatory response to CO_2 and possibly to carbon dioxide retention (Lambertsen 1978). Individuals that retain CO_2 may be more susceptible to CNS oxygen toxicity (Lanphier 1975).

2. METHODS

The study was divided into 10 phases which are summarized in Table 1.

Table 1 - Study Phases

Phase	PI_{O_2} (ATA)	PET_{CO_2} (Torr)	Duration (min)	Immersion (Static Lung Load)	Workload	N
1	0.21 1.75 2.80	40 60	7 (b)	Dry	Rest	34 (a)
2	0.21 1.75 2.80 --- 0.21 1.75 2.80	40 60 --- 40 60	7 (b) --- 7 (b)	Dry --- Head out (c)	Rest	13 --- 13
3	0.21 1.75 2.80	50	10	Dry	Rest	10
4	0.21 1.75 2.80	40	10 40 20	Dry	Rest	11
5	0.21 1.75 2.80	40	10 40 20	Head out (c)	Rest	6
6	0.21 1.75 2.80	40	10 40 20	Total (d)	Rest	6
7	0.21 2.80	40	20 20	Head out (c) Total (d)	Rest	10
8	0.21	40	40	Head out (c)(f) Total (e)(f)	Rest	6
9	0.21 1.75	40	10 40	Dry	Work VO_2 = 1.5 lpm	10
10	0.21 1.75	40	10 40	Total (d)	Work VO_2 = 1.5 lpm	10

Notes:

(a) Included a 7 minute progressive hypoxic rebreathing period prior to CO_2 rebreathing in half of the experiments and all experiments were performed with recumbent subjects while all other phases were performed on subjects seated upright

(b) Rebreathing from normocapnia, denoted as PET_{CO_2}= 40 torr, to PET_{CO_2} = 60 torr required 7 minutes on average

(c) Subjects were immersed to the neck in thermoneutral water (35 °C)

(d) Subjects were fully immersed in thermoneutral water (35 °C) and breathed against a negative static lung load of 10 cm H_2O.

(e) Subjects were fully immersed in thermoneutral water (35 °C) and breathed against a positive static lung load using a modified Mark 15 UBA.

(f) Included a 1 minute breath hold

2.1 Subjects and Safety

This study was approved by the Duke University Institutional Review Board (# 944-92-8R6 latest version, Appendix A). 32 healthy volunteers (28 males, 4 females), ages 18 - 47, gave written informed consent prior to participation in an experiment. Some subjects participated in multiple phases of the study and in multiple experiments within a single phase. Subject characteristics and phase participation are listed in Table 2. Subjects were compensated $100 per experiment. All subjects were required to have had diving physicals including normal chest x-rays during the prior year. Each subject was familiarized with the experimental environment and procedures before testing. Subjects were advised of the nature of CNS oxygen toxicity symptoms and were informed of their right to stop the experiment at any point.

Subjects wore nose clips and breathed the experimental gases through a mouthpiece. They were instructed to remove the mouthpiece and breath air if they became uncomfortable or noted any symptoms that might be oxygen toxicity, namely, tinnitus, tunnel vision, disorientation, muscle twitching, incoordination, light-headedness, anxiety, narcosis, nausea, numbness, and / or tingling. A member of the hyperbaric staff accompanied each subject during an experiment. Subjects wore a safety harness connected to a rope and pulley system and a bicycle helmet to prevent injury in case a convulsion should occur. During immersion studies, subjects held a "dead man switch" which gave off an audible alarm if the subject let go of the switch in case of sudden onset of a convulsion or other problems hidden to the attendant.

To reduce the probability of decompression sickness, the subject and attendant were decompressed on the US Navy Air Decompression Tables (with Duke modifications) for square dives and with 10-25 min of oxygen breathing at 30 fsw after multi-level dives. The multi-level oxygen decompression tables are given in Appendix C.

Table 2 - Subject information

Name	Sex	Age	Phase No(s).
AW	M	32	3,4,5
BFC	M	47	2,4
BC	M	37	4
BW	M	30	2
BL	F	30	7
BP	M	32	9
BB	M	18	3
CW	M	25	4,5
CI	M	38-39	3,4,6,8,9,10
CM	M	33-34	2,3,4,5
DD	F	33	2,3
DL	M	41	7
DV	M	49	2
FC	M	32	3,4,5,6,7,8,9,10
HM	F	21-22	6,7,8,9,10
JB	M	32	7,8,9
JH	M	28	7
JM	M	32	7
JF	M	33	7
JN	M	37	2
KB	M	37	9,10
KS	F	22-23	3,4,5,9,10
MM	M	34	3
MR	M	44-45	2,3,6,7
OD	M	43	2
PF	M	36-37	2,3,4,6,9,10
PD	M	23	9,10
RS	M	39-40	4,6,7,8,9,10
RT	M	32-33	4,5,7,9,10
RF	M	33	7,10
TA	M	32-34	2,8
WW	M	32	2

2.2 Equipment

Experiments were conducted in the upper and lower parts of a hyperbaric chamber. The subject sat on a bicycle ergometer in the lower part which could be filled with water. The attendant and the breathing apparatus were in the upper part.

2.2.1 Spirometer and Breathing Loop

The breathing apparatus was of closed-circuit, recirculating design (Fig. 1) and used a 10 liter rolling dry-seal spirometer (Sensormedics, Model 922) as a counterlung. A potentiometer attached to the piston of the spirometer provided voltage output to the computer. The spirometer was calibrated with a 3 liter syringe (Collins, Model M-20).

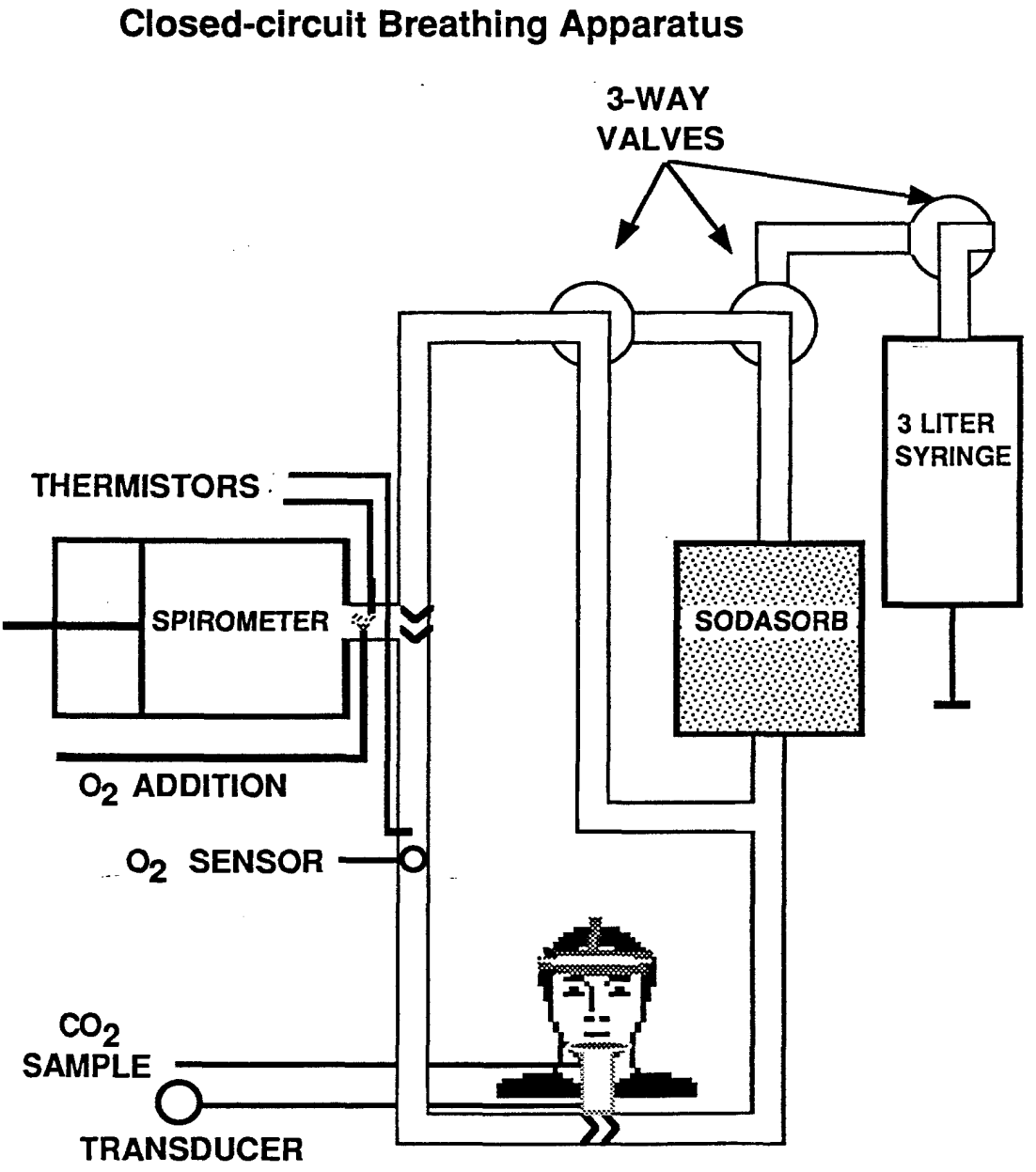

Figure 1: Closed-circuit breathing apparatus used to achieve oxygen and carbon dioxide pressures required for experimental conditions.

A temperature probe (YSI, Model 710) was inserted into the input/output duct of the spirometer to provide data for temperature correction of measured volumes. The thermistor (time constant = 0.3 sec) was calibrated from 17 - 37 °C. Tidal volume (V_T) was corrected to BTPS as follows:

$$V_T = (V_{max} - V_{min}) * T_{Cor} * WV_{Cor}$$

where:

V_{max} = Maximum volume during breath
V_{min} = Minimum volume during breath
T_{Cor} = Temperature correction = $310.0/(273.0 + T_S)$
T_S = Spirometer temperature
WV_{Cor} = $(P_{ata} - PH_2O) / (P_{ata} - PH_2O_{BT})$

where :

P_{ata} = Barometric pressure in ata
PH_2O = Ambient water vapor pressure in ata.
PH_2O_{BT} = Ambient water vapor pressure in ata at body temperature
= 47 / 760 ata

Ambient water vapor pressure was determined, assuming saturation, by using the following third-order polynomial:

$$PH_2O = (2.47488 + (0.65949*T_S) - (0.00712*T_S^2) + (0.00059*T_S^3)) / 760.0$$

This equation was obtained from regression analysis of water vapor pressures at temperatures from 12 to 42°C tabulated in The Handbook of Chemistry and Physics (47^{th} Ed., 1966).

Maximum and minimum volumes were determined using a peak detection software routine to obtain V_T. Respiratory rate was determined by measuring the time between maximum volumes or end-exhalations. Respiratory minute volume (RMV) was calculated as: RMV = V_T x respiratory rate.

Flow was directed into and out of the spirometer via a T-shaped two way valve (Hans Rudolf, Model 2700). This valve provided an entry point for the spirometer temperature probe and an oxygen addition input. Another valve (Hans Rudolf, Model 2700) served as the mouthpiece valve. A silicon rubber SCUBA mouthpiece attached to a microbial filter (Pall

Biomedical, Model P30S) was attached to the valve. These valves were chosen for their low dead space (90 ml), large bore (28.6 mm = 1 $\frac{1}{8}$ in.), and low resistance (0.8 cm H_2O / lpm). Breathing resistance, which increases with density of the breathing gas, was minimized by ensuring that all portions of the breathing loop had an inner diameter (ID) of at least 28.6 mm. The components of the breathing circuit were connected using 35 mm (1 $\frac{3}{8}$ in.) ID smooth bore tubing (Hans Rudolf, Model 9039) .

2.2.2 Computer

Data were recorded on a DEC MINC 11/03 computer on a breath-by-breath basis, triggered at end exhalation. The following variables were sampled at 50 Hz by a 12 bit A/D board installed in the MINC 11/03: inspiratory temperature (°C), expiratory temperature (°C), spirometer temperature (°C), inspiratory oxygen partial pressure (ata), end-tidal CO_2 (torr), volume (L), and peak-peak mouthpiece pressure (cm H_2O). Input voltage range was +5 to -5 volts, allowing 2.44 millivolt resolution. The controlling software was written in Fortran IV. Subroutines were developed for system calibration, oxygen control, and data acquisition, display, and storage.

2.2.3 Oxygen Pressure Control

A micro fuel cell (Teledyne Analytical, Model B7W) was used as an oxygen sensor to control the inspired oxygen partial pressure (PI_{O_2}). This cell had a response time (0 - 90%) of 7 seconds and was mounted in a 1 $\frac{1}{4}$ in. (31.8 mm) copper "T" downstream to the spirometer input/output port. The fuel cell surface was exposed to the gas flow but did not obstruct the breathing loop. The output from the fuel cell was input into a current-voltage converter/amplifier with hardware temperature compensation (YSI, Model 710). The thermistor penetrated the copper "T" in close proximity to the O_2 sensor. The oxygen sensor was calibrated at each depth using Primary Standard calibration gases (National Specialty Gases). Nitrogen was used as the "zero" gas. The "high" calibration gases were 20.90% O_2 at the surface, and 96.00 % O_2 at 35 and 77 fsw.

Oxygen partial pressure control was accomplished by addition of O_2 (99.999% USP O_2) through a computer-controlled solenoid valve connected to a regulator (National Welders, Model HPTD) on a O_2-filled G-cylinder. O_2 flowed from the solenoid through a 60 micron filter and a 0.0135 in. (0.34 mm) orifice designed to achieve a critical flow of 6 liters per minute (Harter, 1967). As the PI_{O_2} dropped below the user defined set point by an input amount, the solenoid was fired for 1.0 second. Comparison of the PI_{O_2} to the set point was done every other breath to allow for gas mixing delays. Computer control was achieved using output from a digital I/O board within the MINC 11/03 computer. To fire the solenoid, the least significant bit of the 8 bit I/O port was set high (+4.5 volts) and sent to the input of a 12 volt solenoid driver.

O_2 flow calibration was performed at each depth by pulsing the solenoid for 1 minute and measuring the increase in volume. Computer software kept track of the amount of time that the solenoid was open and multiplied this duration by the flow rate to determine oxygen addition. Oxygen consumption was calculated by taking a 10 breath moving average of the breath to breath oxygen addition calculations. An average oxygen consumption for a given time period was also calculated. Oxygen addition is equivalent to oxygen consumption in a closed system if the oxygen level is maintained. Errors in oxygen consumption calculation result if oxygen partial pressure control is not tightly maintained, if oxygen partial pressure is not well mixed within the system, or if there are gas leaks into or out of the system. One metabolic source of gas leak into the system is respiratory nitrogen elimination during decompression. Nitrogen absorbed by tissues during air breathing at 77 fsw was "washed out" during oxygen breathing at 35 fsw and necessitated further oxygen delivery to maintain the 1.75 ATA setpoint. Excess volume introduced into the breathing circuit and was eliminated by extracting gas from the system with the calibrated syringe through a three-way valve.

2.2.4 Oxygen Set Points

PI_{O_2}'s of 1.75 and 2.8 were chosen because they are equivalent to 100% oxygen at 25 fsw, the depth of the longest US Navy oxygen exposure limit for working divers, and 100% oxygen at 60 fsw the exposure pressure of US Navy Treatment Tables 5 and 6. Due to nitrogen elimination during oxygen breathing, 100 % oxygen could not be maintained within the breathing circuit. Therefore, 85% oxygen was employed at depths of 35 and 77 fsw to achieve 1.75 and 2.8 ATA PI_{O_2}.

2.2.5 Carbon Dioxide Control

A gas sample was drawn from the mouthpiece valve by the pump in a carbon dioxide analyzer (Beckman, Model LB2) and used to obtain PET_{CO_2} determinations. The gas was sampled at 200 ml/min and returned to the rebreathing circuit at a point in the exhalation side of the spirometer to avoid gas loss due to sampling. The CO_2 analyzer (response time = 0.1 sec from 0 - 90%) was calibrated at each depth using Primary Standard calibration gases, 8.00% CO_2 at the surface, 4.00% CO_2 at 35 fsw, and 2.52% CO_2 at 77 fsw. The output from the analyzer was input into the computer and converted from a surface equivalent percentage to units of torr (8.00% = 60.80 torr at 1 ata) . The CO_2 analyzer was modified to allow a nitrogen purge to be installed proximal to the analyzer's pumps, power supply and infrared source to eliminate the chance of a spark or heat build-up, fire hazards in a hyperbaric chamber. Gas samples were taken from within the analyzer during the compression of the chamber to ensure that less than 4% oxygen remained within the analyzer's electronics compartments. This limited the rate of compression of the chamber to 15 ft/min.

A peak detection software routine was employed to determine the maximum PET_{CO_2} per breath. CO_2 levels were controlled manually by the tender who manipulated two three-way "Y"-shaped stopcocks (Hans Rudolf, Model 4000A, see Fig. 1) in response to changes in the end-tidal CO_2 as indicated by the CO_2 analyzer. The CO_2 level was maintained within 2 torr of a set-point. Use of the stopcocks allowed exhaled flow to pass through a CO_2 absorbent

canister (Collins, Model 21377), by-pass it, or pass partially through and partially around the canister. The canister was filled with Sodasorb (WR Grace) which chemically binds CO_2 via the following reaction (13):

i.) $CO_2 + H_2O \leftrightarrow H_2CO_3$
ii.) $2H_2CO_3 + 2Na^+ + 2OH^- + 2K + 2OH^- \rightarrow 2Na^+ + CO_3^= + 4H_2O$
iii.) $Ca(OH)_2 + H_2O \leftrightarrow Ca^{++} + 2OH^- + H_2O$
iv.) $2Ca^{++} + 4OH^- + 2Na^+ + CO_3^= + 2K^+ + CO_3 \leftrightarrow$
$2CaCO_3 + 2Na^+ + 2OH^- + 2K^+ + 2OH^-$

The reaction is exothermic, yielding 13.5 kcal/gram molecular wt. CO_2, thus heating the exhaled gas. The brief duration (5 - 10 min.) of experiments in phases 2 and 3, prevented large increases in temperature due to the exothermic reaction. Heat build-up did occur in experiments of longer duration (> 10 min), necessitating use of a heat exchanger at the distal end of the Sodasorb canister. The cold air output from a vortex tube (Vortec, Model 106) was directed down copper tubing wrapped around the circuit tubing exiting from the Sodasorb canister and entering the spirometer. This allowed for maintenance of the circuit temperature to below 32° C.

2.2.6 Mouthpiece Pressure

A fitting was placed in the mouthpiece valve to accommodate $\frac{1}{8}$ in. ID tubing connected to a pressure transducer (Validyne, Model MP45-871). The pressure transducer was used to record peak-peak mouthpiece pressure, an indicator of breathing resistance (14). The mouthpiece pressure was calibrated from 0 - 40 cm H_2O pressure using an electronic manometer (Setra Systems Inc., Model 339-1).

2.2.7 Heart Rate

Heart rate was monitored by one of two methods which were tested against each other and provided comparable results. Initially, a telemetry system (Polar Accurex) was utilized. The transmitter was attached to an elastic band worn on the chest and the receiver was a

wristwatch display positioned in view of the attendant. Heart rate was recorded by the attendant at two minute intervals. Later ECG electrodes were attached to the subject and the subjects was connected to an ECG monitor (PhysioControl, Model VSM-1).

2.2.8 Bicycle ergometer

A bicycle was constructed of aluminum and stainless steel parts to prevent corrosion during immersion studies. The bicycle was outfitted with a cycle computer (Cateye Micro cyclocomputer Model cc 6000) which included a time and rpm (revolutions per minute) display which was located at the subject's eye level. The bicycle was not a true ergometer. The subject was instructed by the chamber attendant to maintain a rpm for a given duration. Modification of the goal rpm was relayed to the subject by the attendant based upon the average oxygen consumption for the period of interest. During dry studies, some subjects had difficulty maintaining slow enough rpm to produce 1500 ml / min oxygen consumptions and were put on 6 / 4 min work / rest cycles to achieve an average oxygen consumption of 1500 ml / min.

2.2.9 NIRS Instrument

The near-infrared spectroscopy (NIR) instrument employed during this study uses laser diodes as monochromatic light sources. Laser diodes have narrow bandwidths (1.5 nm) and can be pulsed rapidly in succession for multi-wavelength applications. These are p-n junction devices optimized for radiant output. The Ga-Al-As diodes used have high band gaps resulting in wavelengths in the NIR, 775, 810, 870, and 904 nm. Using time-domain multiplexing, the laser diodes are pulsed at a frequency of 1 KHz with a pulse width of 200 ns.

The near-infrared light is transmitted and recovered through two optical fiber bundles called optrodes. Non-coherent optical fiber bundles are used to prevent bias due to spatial illumination differences. Both optrodes were potted into brass penetrators, allowing for their use in the hyperbaric chamber. The optrodes used are 4 meters in length. At their ends

inside the chamber, the optical fibers were potted into aluminum terminators 1 cm in diameter with polished ends. These terminators allow the optrodes to be securely fastened to a head band which positions the centers of the fiber bundle terminators 3.5 cm apart at an angle of approximately 30 degrees. The head band was positioned to ensure that the optical fiber bundles were directed away from the frontal sinus. The band was tightened to blanch the skin of blood flow beneath the optrodes. The optical fiber bundles were kept in the same place on the subject's forehead throughout the entire experiment in order to allow comparison of the signals for each condition. Exact placement of the optrodes was determined by adjusting the headband until a strong stable signal was acquired.

Photons enter the tissue after passing through the skin and bone, travel a distance via multiple reflection and scattering, and emanate at different distances from the entry point. In tissue, photons of selected wavelengths are absorbed by the copper atoms of cytochrome a,a_3 (Cyt a,a_3) and the iron-porphyrin complexes of tHb and $tHbO_2$. Changes in the oxidation reduction state of Cyt a,a_3 and concentrations of tHb and $tHbO_2$ in the illuminated area can be detected as changes in the amount of reflected light. A fraction of the reflected photons are captured by the receiving optrode and conveyed to the detector. Some optical fibers in the transmitting optrode are designated to carry back scattered incident light to a separate detector as a reference signal since absorbence is measured as a ratio of incident light intensity to collected light intensity. The transmitting fiber optic bundle is therefore split into five legs at the instrument, one carrying back scattered light to the reference photodiode, and the four others carrying light from the four laser diodes to the tissue. The instrument end of the receiving optrode terminates as an aluminum block fitted to interface with a photomultiplier (Hamamatsu model R936). All connections are made using mechanical fasteners and optical coupling gel (Math Associates Inc.) is placed at all interfaces including the skin/optrode interface.

The photocurrents from the two photodetectors, the reference photodiode and the signal photomultiplier, are integrated, demultiplexed, and fed through a log ratio amplifier. The log

signal/reference voltages are then input to a dedicated microprocessor, where algorithms are applied and the metabolic signals are displayed in real time (10 second time constant) on a printer (Epson model FX 100).

NIRS validation experiments were done using fluorocarbon emulsion, FC-43, exchange transfusion in cats and rats. These experiments verified that algorithms and instrumentation used determine relative changes in the concentrations of Hb, HbO_2, and cytochrome a,a_3 in the illuminated area accurately and independently (Piantadosi, 1989b).

2.3 Experimental Protocols

Experiments began with placement of the nose clips and initiation of normal breathing through the mouthpiece. The rebreather was filled with air, 0.21 ATA P_{IO_2}, to start each experiment. Normocapnic normoxia was maintained for 5 minutes while the subject was dry and seated at rest. This period served as the control for each experiment unless otherwise noted. When multiple hyperoxic exposures were to occur on the same day, after the 0.21 ATA P_{IO_2} exposure, the chamber was compressed to 77 fsw to do the 2.80 ATA P_{IO_2} exposure and then decompressed to 35 fsw to do the 1.75 ATA P_{IO_2} exposure. These depths and the order of exposures were chosen to minimize the risk of decompression sickness to the subject and inside attendant. A minimum of 20 minutes recovery took place between any two exposures. Calibration at depth usually required 15 minutes. Compression to 77 fsw took 5 min to allow for adequate purging of electrical equipment.

2.3.1 Phase 1 - CO_2 Ramp Study - Recumbent

Phase 1 studies were performed to measure the effect of elevated inspired CO_2 during hyperoxic breathing on cerebral oxygenation, ventilation, and symptom incidence. Phase 1 studies were performed on dry, resting, recumbent subjects. During subsequent experiments, subjects sat upright on a bicycle ergometer to ensure consistency for wet, dry, working and resting studies.

The closed-circuit breathing apparatus of Fig. 1 was used to manipulate CO_2 content and maintain P_{IO_2}'s of 0.11, 0.21, 1.75 and 2.80 ATA. The hypoxic exposure, P_{IO_2} = 0.11 ATA, was conducted in half of the experiments in this phase to compare the performance of the NIRS instrument to data from a hypoxia study done earlier using the same instrument but different optrodes (Hampson et al, 1990). The hypoxic exposure was done by having the subject breathe on the closed-circuit rebreathing system without addition of oxygen until the subject's S_aO_2 reached 70% as measured with a pulse oximeter (Nellcor, Model N-100). The rebreathing process took approximately 7 minutes and the average P_{IO_2} was 0.11 ATA. Half of the experiments in this phase were not preceded by the hypoxic exposure to determine if the hypoxia had any effect on subsequent measurements.

The CO_2 ramp, which refers to progressive hypercapnia to 60 torr $P_{ET}CO_2$, was used to measure ventilatory response to CO_2. This was achieved by directing the flow within the breathing circuit through a by-pass, around the Sodasorb canister. By-passing the CO_2 absorbent allowed inspired and end-tidal CO_2 to rise within the rebreathing system. After 60 torr $P_{ET}CO_2$ was achieved, exhaled flow was diverted through the Sodasorb canister until $P_{ET}CO_2$ reached it's control level and was maintained for 2 minutes. After doing the CO_2 ramp with the P_{IO_2} = 0.21 ATA, the exposure was repeated at 2.80 and then 1.75 ATA P_{IO_2}.

2.3.2 Phase 2 - CO_2 Ramp - Seated - Wet and Dry

Phase 2 repeated the experimental conditions of Phase 1 with the following exceptions: subjects were seated upright on a bicycle ergometer, no hypoxic exposures were done, and each dry experiment was repeated with immersion to the neck in thermoneutral water (35 °C). The dry portion of this phase was repeated to investigate the effect of position on the measurements taken. The subject position was changed to accommodate the immersion and work portions of the study. Wet and dry experiments were performed on different days.

2.3.3 Phase 3 - CO_2 exposure duration

Phase 3 experiments were conducted to investigate the effect of a constant elevated inspired CO_2 for a ten minute duration. Progressive hypercapnia to 50 torr $P_{ET}CO_2$ was

achieved by directing flow within the breathing circuit to a by-pass around the Sodasorb canister. Once 50 torr $P_{ET_{CO_2}}$ was reached, flow was split, partially through the Sodasorb canister and partially through the by-pass. This split flow allowed the CO_2 level to be maintained at 50 torr $P_{ET_{CO_2}}$ for 10 min. After 10 minutes, flow was diverted fully through the Sodasorb canister until $P_{ET_{CO_2}}$ reached it's control level and was maintained for 2 minutes. Subjects were dry. All other experimental conditions and procedures were the same as those used in Phase 2.

2.3.4 Phase 4 - Hyperoxic Exposure Duration - Dry

Phase 4 experiments were conducted to investigate the effect of hyperoxic exposure duration on cerebral oxygenation, ventilation, and symptom incidence in dry, resting subjects. Due to the length of the oxygen duration studies (20 min at 77 fsw and 40 min at 35 fsw) , experiments using 1.75 and 2.80 ATA $P_{I_{O_2}}$, were done on separate days. The subject breathed 0.21 ATA $P_{I_{O_2}}$ for 10 minutes at the surface to obtain control data. The subject was compressed to either 35 or 77 fsw, the breathing circuit was filled to and maintained at 1.75 or 2.80 ATA of oxygen and CO_2 was scrubbed for the duration of the experiment. The subject repeated the experiment at least 24 hours later at the other $P_{I_{O_2}}$.

2.3.5 Phase 5 - Head-out Immersion

Phase 5 experiments were conducted to investigate the effect of head-out immersion during hyperoxic exposure on cerebral oxygenation, ventilation, and symptom incidence. Phase 4 was repeated with the subject immersed to the neck in thermoneutral (35° C) water.

2.3.6 Phase 6 - Total Immersion

Phase 6 experiments were conducted to investigate the effect of total immersion during hyperoxic exposure on cerebral oxygenation, ventilation, and symptom incidence. Phase 5 was repeated with the subject completely immersed in thermoneutral water. Swim goggles were worn during all total immersion exposures.

2.3.7 Phase 7 - Facial Immersion

Phase 7 was done to compare head-out and total immersion directly and investigate the effect of facial immersion on cerebral oxygenation, ventilation, and symptom incidence. Subjects initially breathed 0.21 ATA PI_{O_2} at the surface immersed to the neck for 10 minutes. The water was then raised above their heads for 10 minutes and then brought back to the initial head out level. This was repeated at 2.80 ATA PI_{O_2}. The head-out condition was used as the control condition to which NIRS measurements were referenced.

2.3.8 Phase 8 - Static Lung Load and the Dive Reflex

Phase 8 was conducted to test the effect of static lung load on cerebral oxygenation. Total immersion created a negative static lung load on the subjects as the compliant volume was above the water level and the lungs below. This created approximately -30 cm H_2O of static lung load. To test if negative static lung load had an effect on the cerebral oxygen measurements made throughout the study, a Mark 15 UBA was modified to be used in the breathing circuit. The compliant volume of the breathing circuit was then at the level of the lungs. A positive pressure was maintained in the breathing circuit to ensure adequate breathing volume. Subjects breathed 0.21 ATA PI_{O_2} from the modified circuit immersed to the neck for 10 minutes. The head-out condition was used as the control condition to which NIRS measurements were referenced.

To determine the contribution of the dive reflex to measurements taken during facial immersion, breath holds were performed. In the first minute of the head out condition, a 1 minute breath hold was performed. The water was the raised above the subjects heads for 10 minutes and then back to the initial level. After a 10 minute recovery period, the water was again raised above the subject's head. After 5 minutes a one minute breath hold was performed. The water was then brought back to the original head out level. Heart rate was recorded via ECG monitor during this phase of the study. The head-out condition was used as the control condition to which NIRS measurements were referenced.

2.3.9 Phase 9 - Dry Work

Phase 9 was a repeat of Phase 4 oxygen duration during dry rest except that the subjects performed light exercise (1.5 lpm VO_2) and only 0.21 and 1.75 ATA PI_{O_2} exposures were done. VO_2 was measured as a moving average over 10 breaths based on the amount of oxygen added to the breathing circuit. Exercise level was achieved through verbal instruction via the inside chamber tender as to the rpm of the bicycle ergometer as measured by a cyclocomputer. Some subjects performed a work/rest cycle (6/4 minutes) to achieve the required average work load. Heart rate was monitored via telemetry.

2.3.10 Phase 10 - Totally Immersed Work

Phase 10 was a repeat of Phase 9 except that subjects were completely immersed in thermoneutral water. Heart rate was monitored via telemetry.

2.4 Data Analysis

2.4.1 Cerebral Oxygenation

The NIR instrument provides assessment of cerebrocortical oxygenation by monitoring relative changes in deoxygenated hemoglobin (tHb), oxygenated hemoglobin ($tHbO_2$), the sum of tHb and $tHbO_2$ or blood volume (tBV), and the concentration of oxidized Cyt a,a_3. Changes in tHb, $tHbO_2$, and tBV relate to changes in small cerebral blood vessels and while changes in the redox state of Cyt a,a_3 pertain to the frontal parietal cortex. These measures are expressed as differences from normoxic, normocapnic, normobaric control values for each subject. Quantification of the concentration of the optically active species is prohibited by the inability to measure optical pathlength. The NIR data are therefore reported as variations in optical density (vd) from baseline. Variations in density are proportional to changes in concentration. Optical data were averaged over 30 sec intervals and are reported as changes in v.d. (Δvd) from baseline for each parameter. Figure 2 shows an example of signals recorded by the NIR instrument during a hypercapnic exposure in Phase 2.

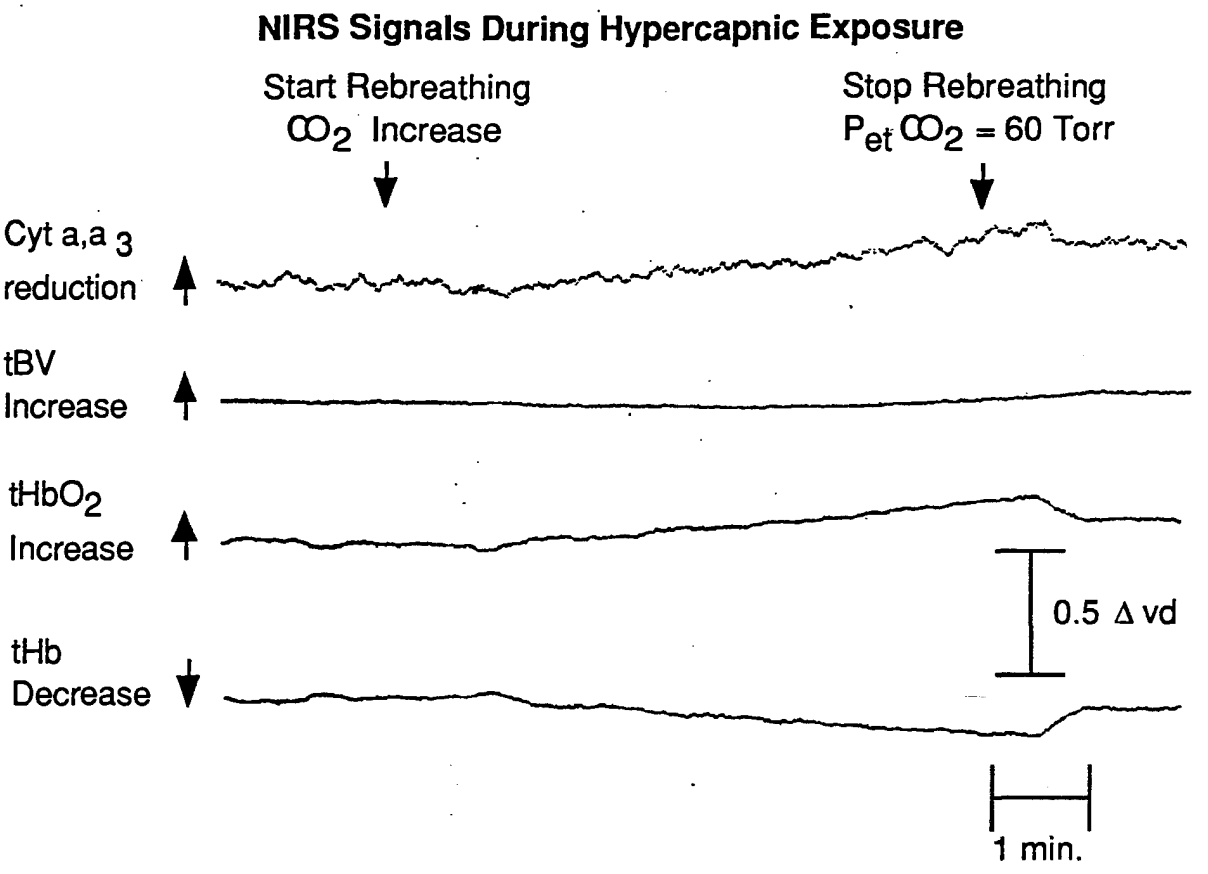

Figure 2: NIRS signals during hypercapnic exposureto 60 torr PET_{CO_2} at PI_{O_2} = 0.21 ATA.

2.4.2 Ventilatory Response

Ventilatory response data is given as the slope of the linear regression of respiratory minute volume (RMV) as a function of PET_{CO_2} from 45 to 60 torr (Figure 3). Data ere imported to Cricket Graph ver. 1.3.1 (Cricket Software) on a Macintosh computer, where the data was graphed and linear regression performed.

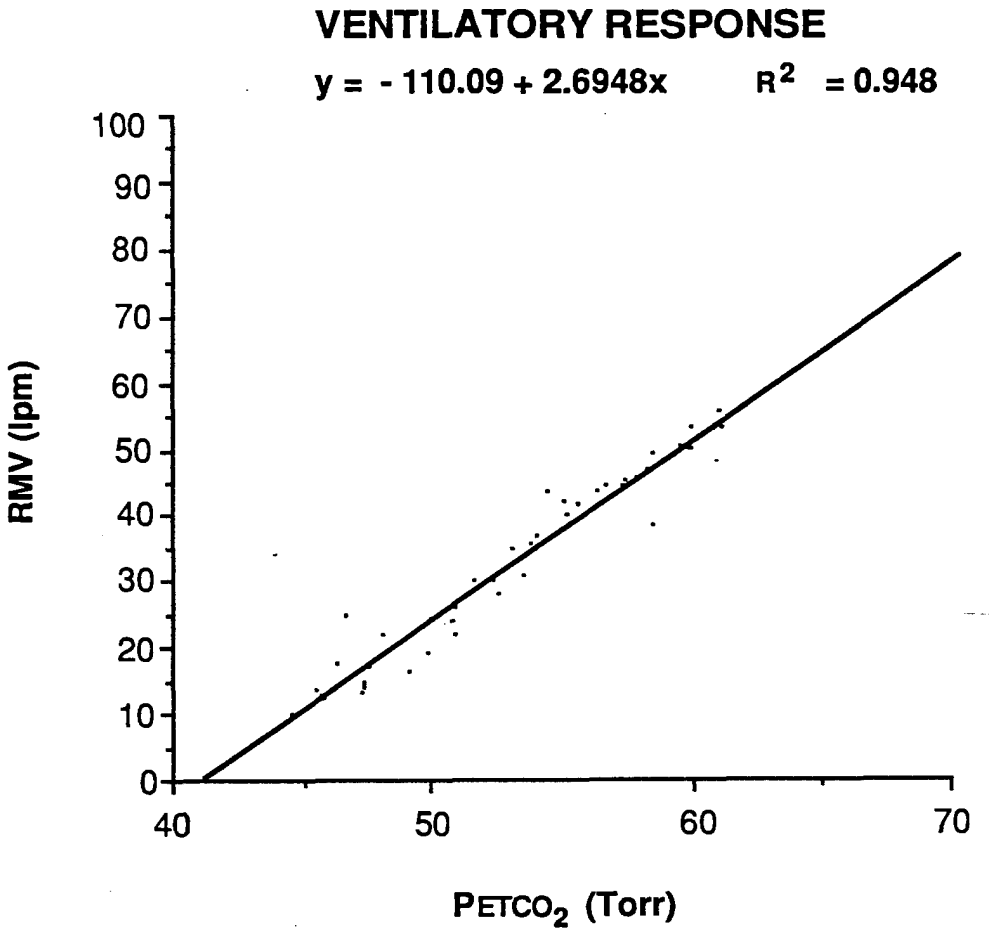

Figure 3: Ventilatory response to CO_2 determination from data recorded during a hypercapnic exposure at 0.21 ATA PI_{O_2}.

2.5 Statistical Methods

Cerebral oxygenation, ventilation, and other data were summarized by calculating mean ± standard deviation (SD) for subjects undergoing the same phases of the study. Participant numbers are listed in Table 2. Significant differences, $p < 0.05$, between experiments grouped by condition were identified utilizing paired and unpaired t-tests employed by using Statview on a Macintosh computer.

3. RESULTS

3.1 Phase 1 - CO_2 Ramp Study - Recumbent

The results of Phase 1 are reported in detail in Appendix A. Inclusion of an hypoxic exposure prior to CO_2 studies had no significant effect on ventilatory response, cerebral oxygenation, or symptom occurrence. The cerebral oxygenation data obtained from the hypoxic exposure in 17 experiments compared favorably with the earlier study done with the same machine but different optrodes (Hampson, 1990). Based on this comparison, the NIRS instrument was used in all following Phases of the study and the hypoxic exposure was no longer included.

Phase 1 investigated the effects of hyperoxia and hypercapnia on ventilatory response to CO_2, cerebral oxygenation, and symptoms in 34 experiments involving 11 subjects. Definite symptoms (tunnel vision, ringing in the ears, and irritability) associated with CNS oxygen toxicity were reported within 3 torr of the maximum $P_{ET}CO_2$ at 2.80 ATA P_{IO_2} in 6 of 34 experiments. Another 15 minor symptoms (tingling, numbness, narcosis, sweats, and dizziness) were reported under the same conditions. All subjects with definite symptoms and 7 of 10 with minor symptoms showed low ventilatory response slopes (ventilation vs. $P_{ET}CO_2$) at 2.80 ATA P_{IO_2} relative to normoxic slopes. Cerebrocortical blood volume relative to normoxia, decreased in the overall group with increasing P_{IO_2}. CO_2 rebreathing reversed this effect such that relative cortical blood volume was significantly greater ($p<.0001$) at a $P_{ET}CO_2$ of 60 torr than at normocapnia for all P_{IO_2}'s tested. Despite the blood volume response to CO_2, the amount of oxidized cytochrome $\underline{a},\underline{a}_3$ did not change significantly. Experiments in which symptoms were reported resulted in smaller blood volume increased at maximum $P_{ET}CO_2$ for a P_{IO_2} of 2.80 ATA than at 0.21 ATA P_{IO_2} (and a tendency for the amount of oxidized cytochrome to increase).

3.2 Phase 2 - CO_2 Ramp Studies

Phase 2 was similar to Phase 1 except that the subjects were seated upright on a bicycle ergometer, and there was no hypoxic exposure. During an additional arm of Phase 2, subjects were immersed to the neck in thermoneutral water (35° C). Eleven subjects, 10 male and 1 female, mean (±SD) age = 37.6 (± 6.6) completed 13 experiments.

3.2.1 Ventilatory Response

Ventilatory response to CO_2 decreased significantly with increasing PIO_2 (Figure 4).

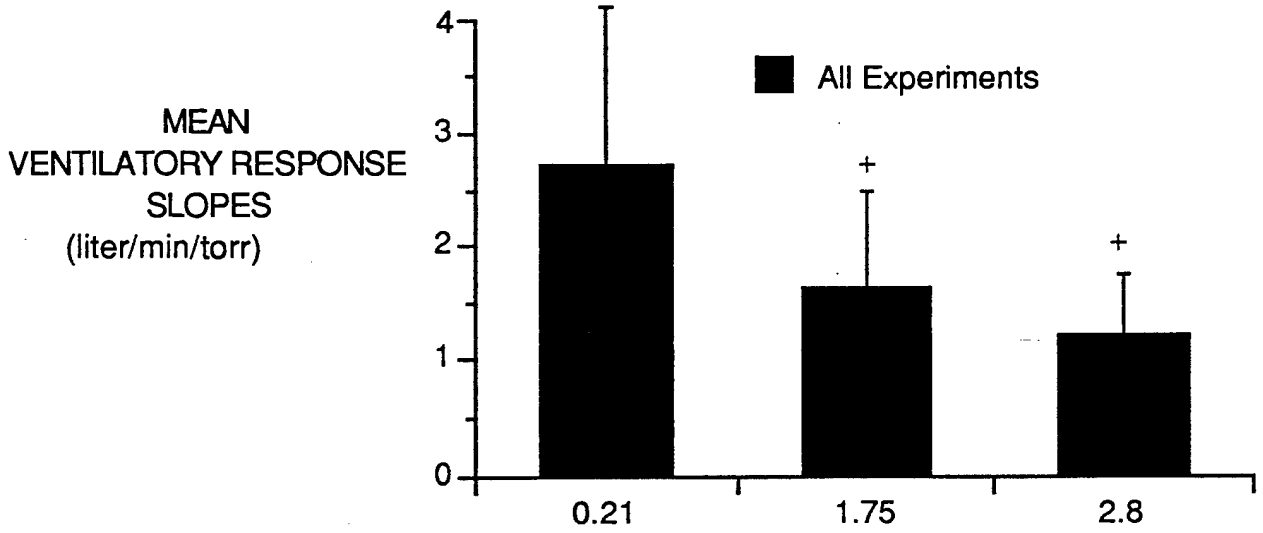

Figure 4: Mean ventilatory response slopes for all experiments. + Sig. diff. from the 0.21 ATA PIO_2, $p < 0.05$.

Ventilatory response to CO_2 did not change with head-out immersion (Figure 5).

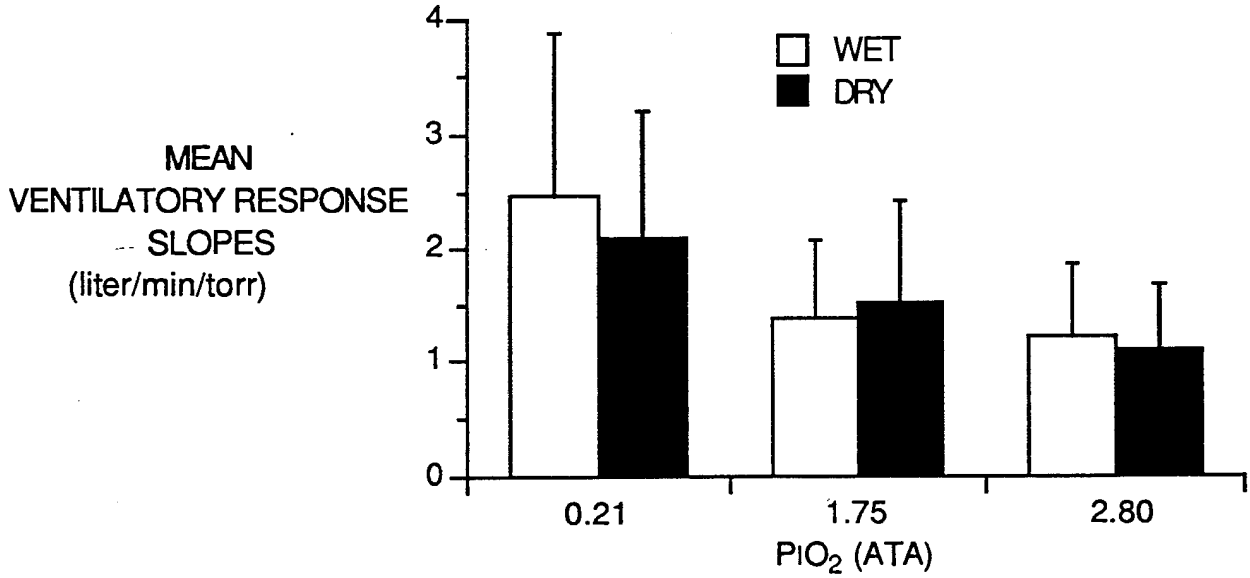

Figure 5 : Mean ventilatory response slopes for wet and dry experiments . + Sig. diff. from 0.21 ATA PIO_2, $p < 0.05$. * Sig. diff. from wet experiments, $p < 0.05$.

Ventilatory response to CO_2 was not significantly different between the group which exhibited symptoms and the group without symptoms (Figures 6 and 7).

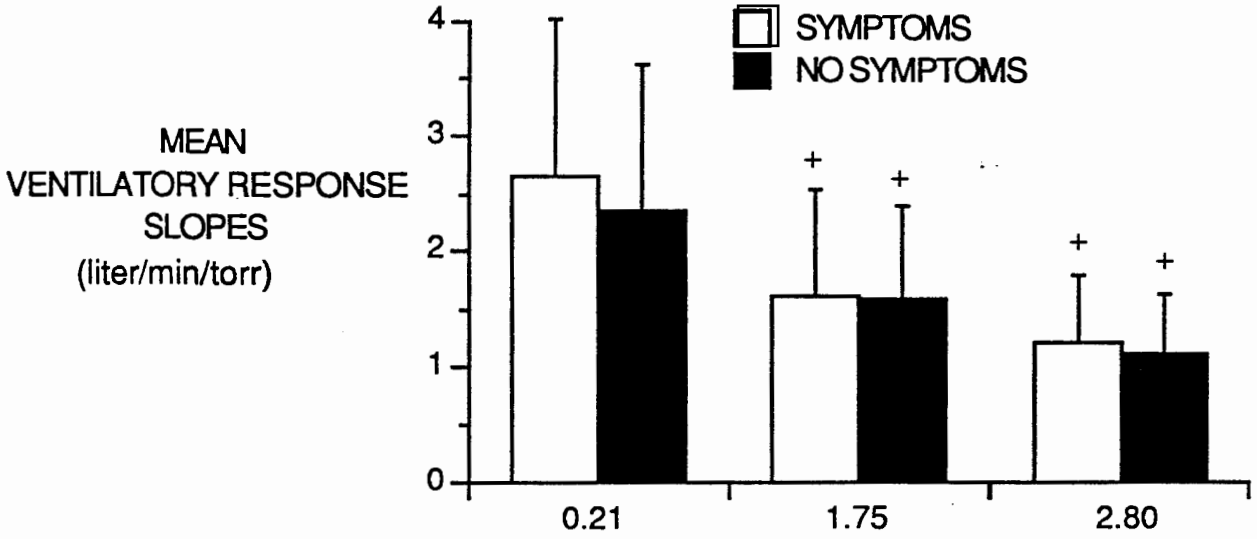

Figure 6 : Mean ventilatory response slopes for subjects with and without symptoms. + Sig. diff. from 0.21 ATA PIO_2, $p < 0.05$. * Sig. diff. from the group w/symptoms, $p < 0.05$.

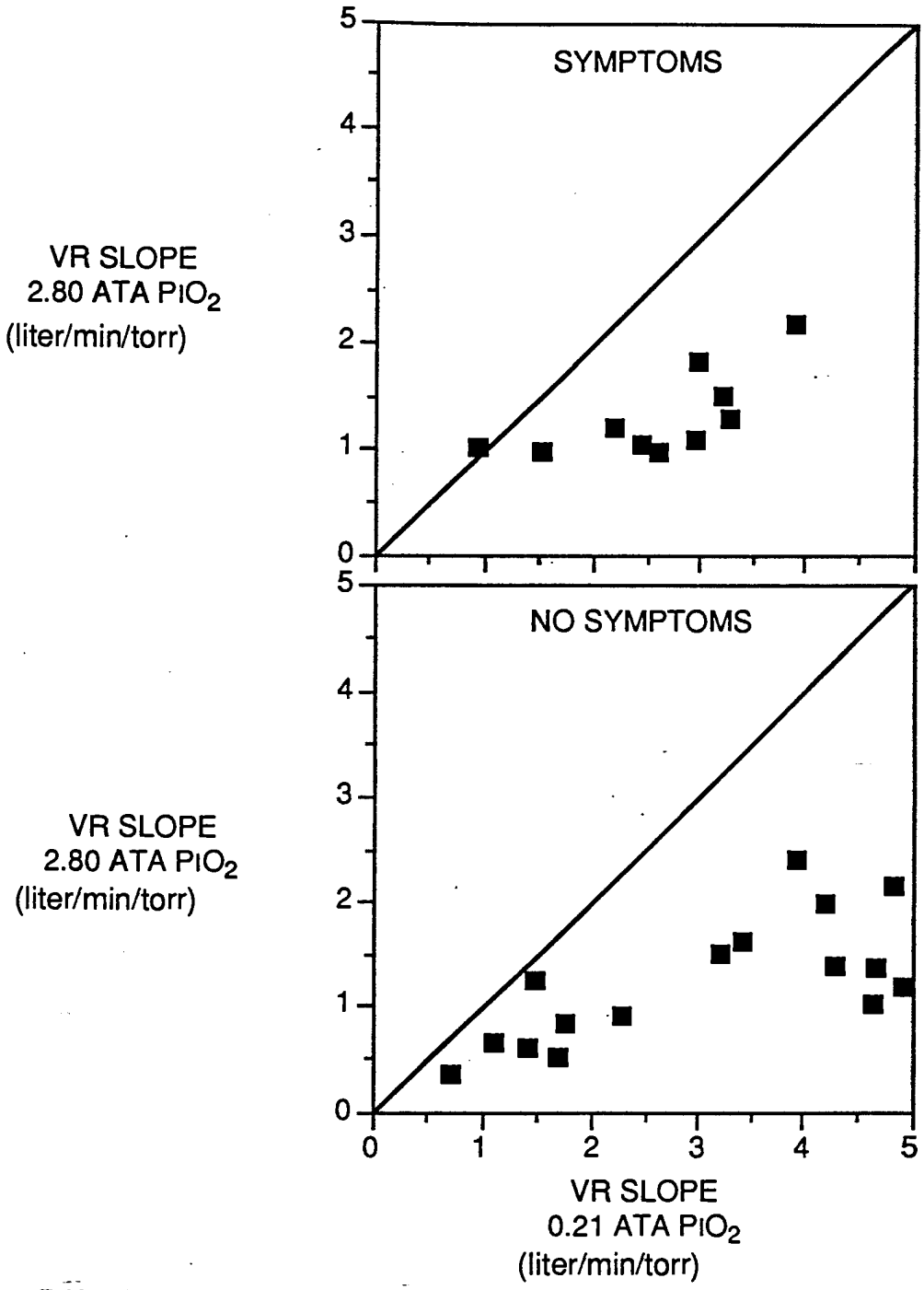

Figure 7: Ventilatory response slopes at 0.21 and 2.80 ATA P_{IO_2} grouped by symptom occurrence.

Duration, the time required for the $P_{ET_{CO_2}}$ to rise for 0 torr to 60 torr, varied between subjects and with P_{IO_2}. Because the 2.80 ATA P_{IO_2} exposure occurred before the 1.75 ATA P_{IO_2} exposure, nitrogen elimination occurred during the latter, increasing overall volume. This slowed the rise of inspired CO_2 and thus slowed the rise of $P_{ET_{CO_2}}$ (Figure 8). Duration was not different when split by symptom groups, but was significantly longer during wet 0.21 and 1.75 ATA P_{IO_2} exposures compared to dry exposures at those O_2 levels.

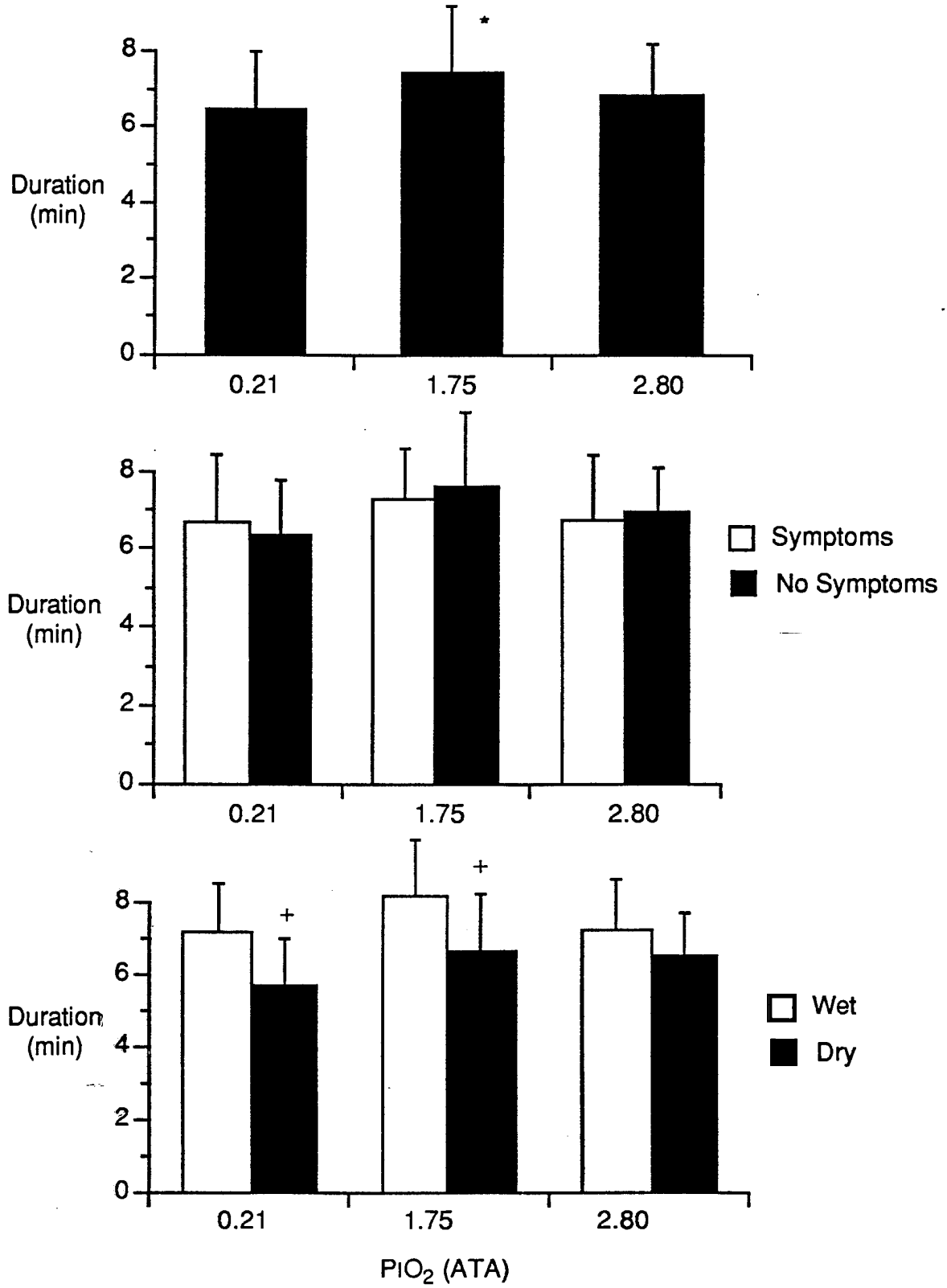

Figure 8: Duration of CO_2 rebreathing for all experiments (top), by symptom occurrence (middle), and by wet/dry (bottom). * Sig. diff. from 0.21 ATA PIO2, $p < 0.05$. + Sig. diff. from the symptom/wet group, $p < 0.05$.

3.2.2 Cerebral Oxygenation

Rebreathing CO_2 to 60 torr $P_{ET}CO_2$ increased $tHbO_2$, tBV, and oxidized cyt. a,a_3 while tHb decreased as shown in a typical experiment in Figure 9.

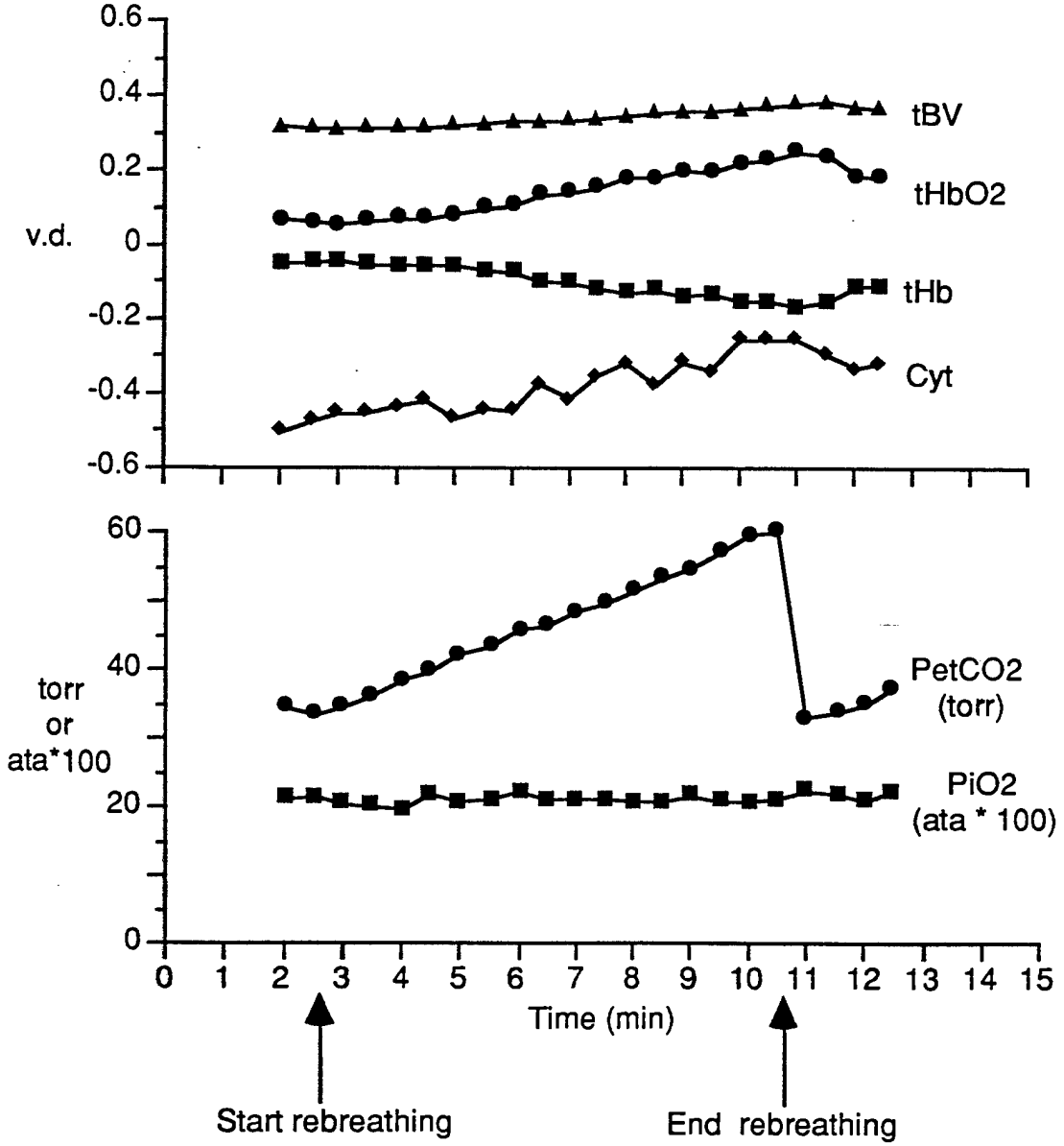

Figure 9: Typical CO_2 ramp experiment. Cerebral oxygenation measurements - brain tHb, tHbO2, tBV, and oxidized Cyt a,a3 and PIO2 and PETCO2 are shown for a normoxic, resting, dry subject.

Mean cerebral oxygenation data are expressed as changes in NIR signals from control. Control readings were taken after 5 minutes of normoxic, normocapnic, normobaric breathing. Changes from baseline were calculated after 5 minutes of breathing gas mixtures with no inspired CO_2 at each P_{IO_2} setpoint and again when $P_{ET}CO_2$ reached 60 torr during

CO_2 rebreathing. Head out immersion in thermoneutral water had no significant effect on cerebral oxygenation as seen in Figure 10 below.

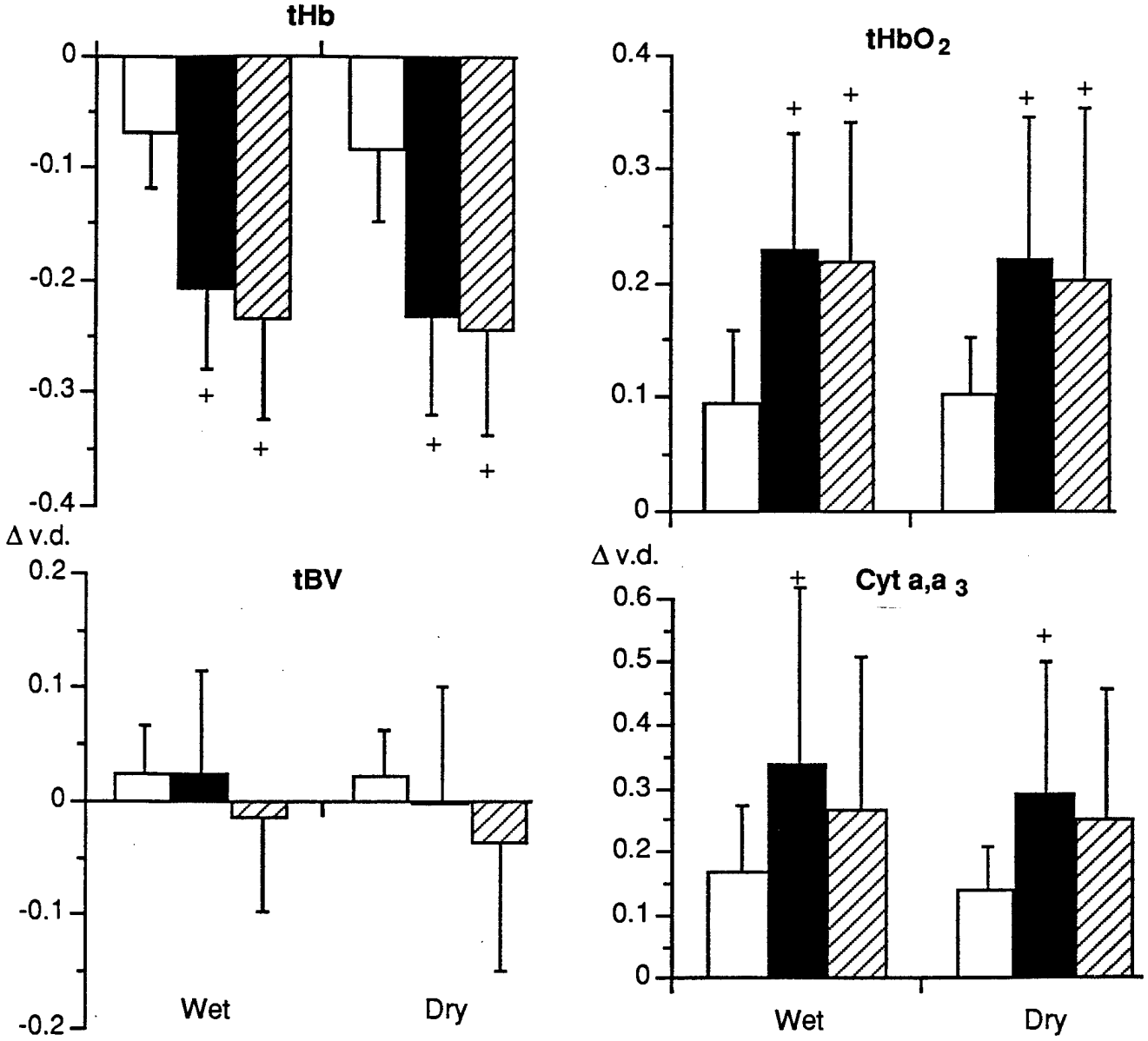

Figure 10: Mean cerebral oxygenation changes for dry and head out immersed CO_2 ramp studies. Changes in variations in density (Δvd) from control in brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 at maximum CO_2 in wet and dry experiments at 0.21 (□), 1.75 (■), and 2.80 (▨) ata PI_{O_2}. Mean ± SD for 13 experiments. * Sig. diff. from wet, $P < 0.05$. + Sig. diff. from 0.21 ATA PI_{O_2}, $P < 0.05$.

Mean data from all experiments are shown in Figure 6. Increased inspired oxygen partial pressure increased $tHbO_2$ and oxidized cyt. a,a_3 while decreasing tHb. Brain blood volume showed a trend toward vasoconstriction but was not a significant result. Rebreathing to 60 torr PET_{CO_2} increased $tHbO_2$, tBV, and oxidized cyt. a,a_3 while decreasing tHb at each PI_{O_2} with one exception. No blood volume increase was observed at 2.80 ata PI_{O_2}.

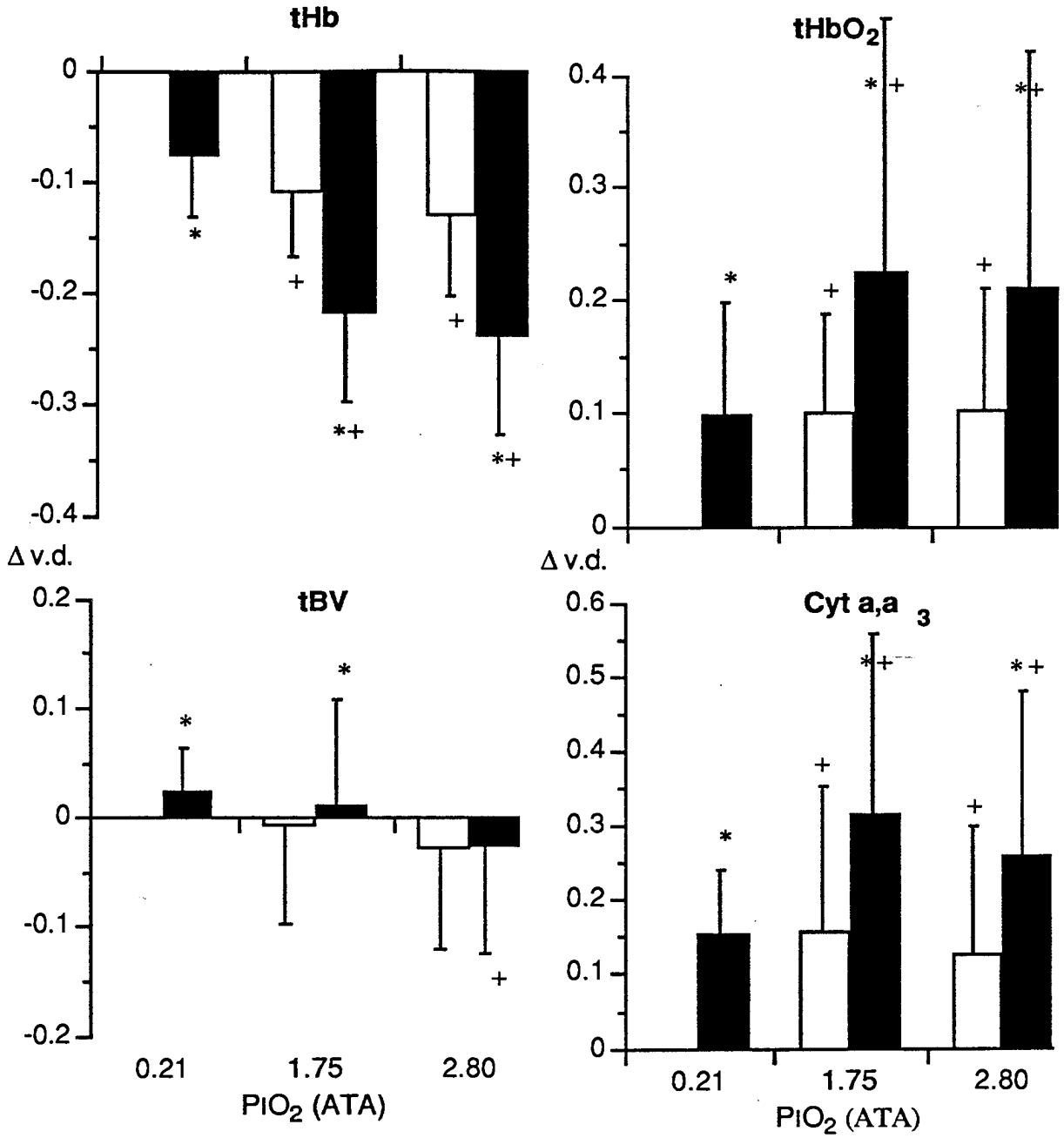

Figure 11: Mean cerebral oxygenation changes for combined wet and dry CO_2 ramp experiments. Changes in variations in density (Dvd) from control in brain tHb, tHbO2, tBV, and oxidized Cyt a,a_3 in subjects breathing no CO_2 (□) and maximum CO_2 (■), 60 torr $PETCO_2$, at different inspired oxygen pressures. Mean ± SD for 26 experiments. * Significantly different from the no inspired CO_2 value, $P < 0.05$. + Significantly different from 0.21 ata PIO_2 value, $P < 0.05$.

Except for changes in blood volume, there were no differences in cerebral oxygenation between subjects with symptoms and those without. Subjects with symptoms had smaller

increases in blood volume during normoxic breathing at maximum CO_2 than subjects without symptoms (see Figure 12).

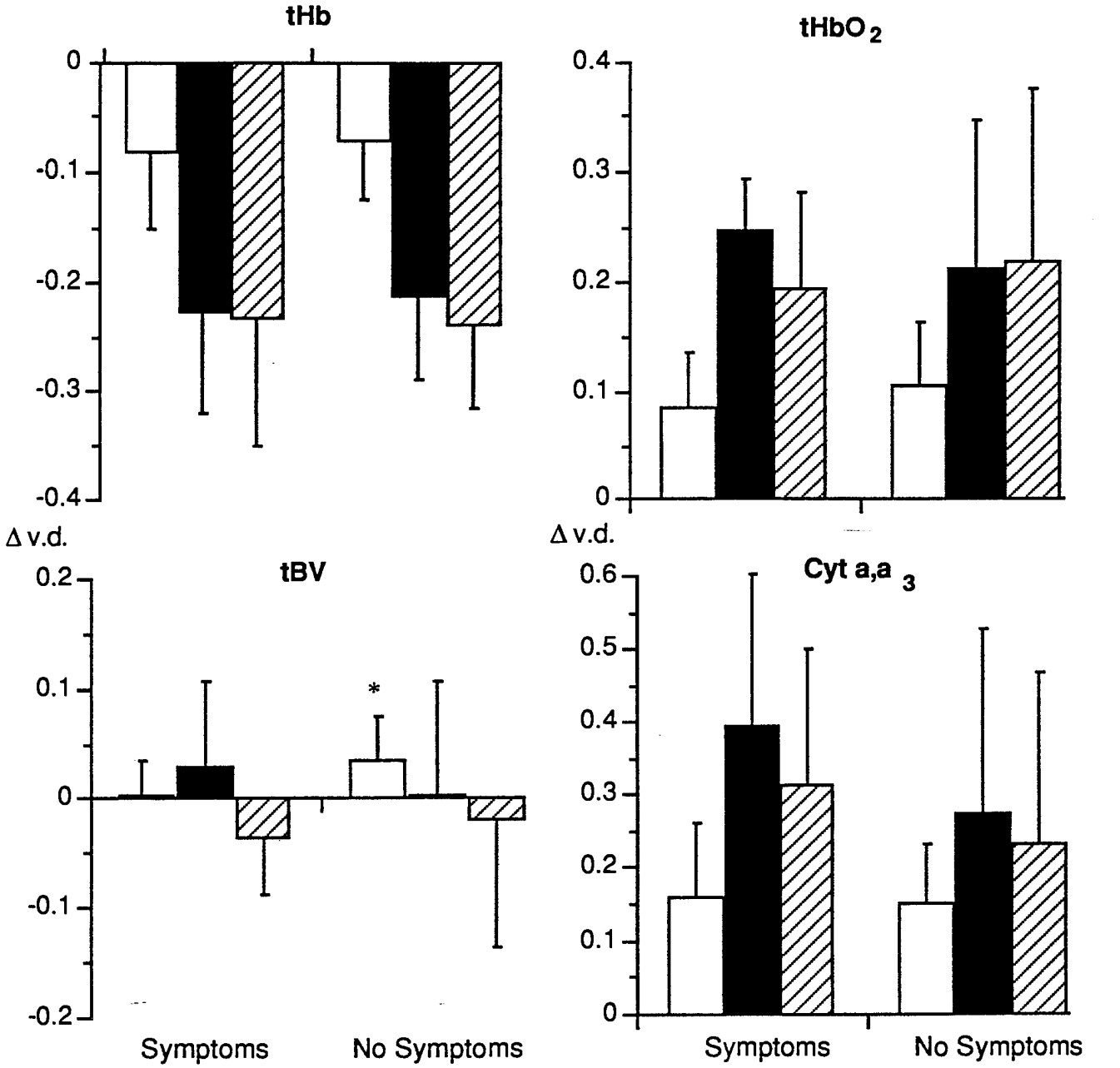

Figure 12: Mean cerebral oxygenation changes. Changes in variations in density (Δvd) from control in brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 in experiments with reported symptoms (n=10) and those without reported symptoms(n=16) at maximum CO_2 and 0.21 (□), 1.75 (■), and 2.80 (▨) ata PI_{O_2}. Mean ± SD for 26 experiments.

* Significantly different from the symptom group, $P < 0.05$.

3.2.3 Symptoms

Subjects reported symptoms consistent with mild CNS oxygen toxicity in 10 of 26 experiments. The symptoms are summarized Table 3. No subjects reported multiple symptoms and symptoms were only reported during the 2.80 ATA P_{IO_2} exposure. All symptoms were reported within 3 torr of the 60 torr $P_{ET_{CO_2}}$ endpoint at a P_{IO_2} of 2.80 ATA. Symptoms abated upon decrease of inspired CO_2. Head out immersion in thermoneutral water had no apparent effect on symptom occurrence, 5/13 subjects reported symptoms for both wet and dry conditions.

Table 3 - Symptoms

Symptom	Dry	Wet
Facial twitching	1	2
Ringing in ears	0	2
Nausea	0	1
Extreme anxiety	1	0
Nausea	1	0
Narcosis	1	0
Light headed	1	0

3.3 Phase 3 - CO_2 Duration Studies

Nine subjects, 7 male and 2 female, mean (±SD) age = 32.3 (±7.6) were tested in 10 experiments. 9 experiments were completed and one experiment was interrupted when a male subject, age 34, had a convulsion.

3.3.4 Ventilatory Parameters

The subject who convulsed had the lowest ventilatory response to CO_2 on the surface and at 2.80 ATA P_{IO_2}. The subject with mild symptoms had a high ventilatory response at 0.21 ATA and a mid-range response at 2.80 ATA P_{IO_2} (Figure 13).

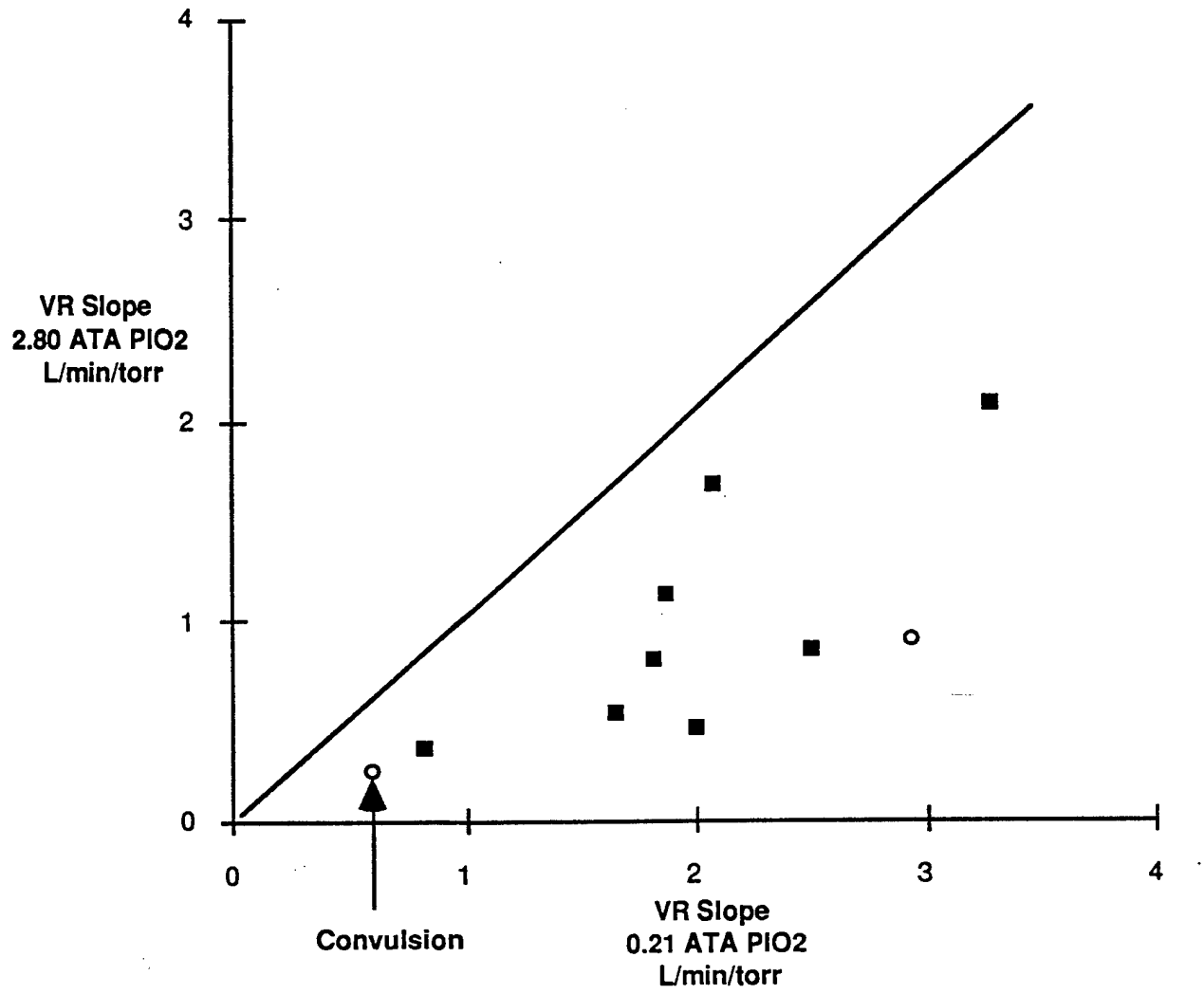

Figure 13 : Ventilatory response slopes from 0.21 ATA and 2.80 ATA P_{IO_2} experiments . The subjects with symptoms are designated by circles and the subject with the convulsion is indicated by the arrow.

Ventilation prior to CO_2 rebreathing did not change significantly with increasing P_{IO_2}. Ventilation at the end of the 10 minute rebreathing period showed a significant decrease with increasing P_{IO_2}.

Ventilation was significantly higher at the end of each rebreathing period compared to ventilation prior to rebreathing, normocapnia, at each P_{IO_2} (Figure 14).

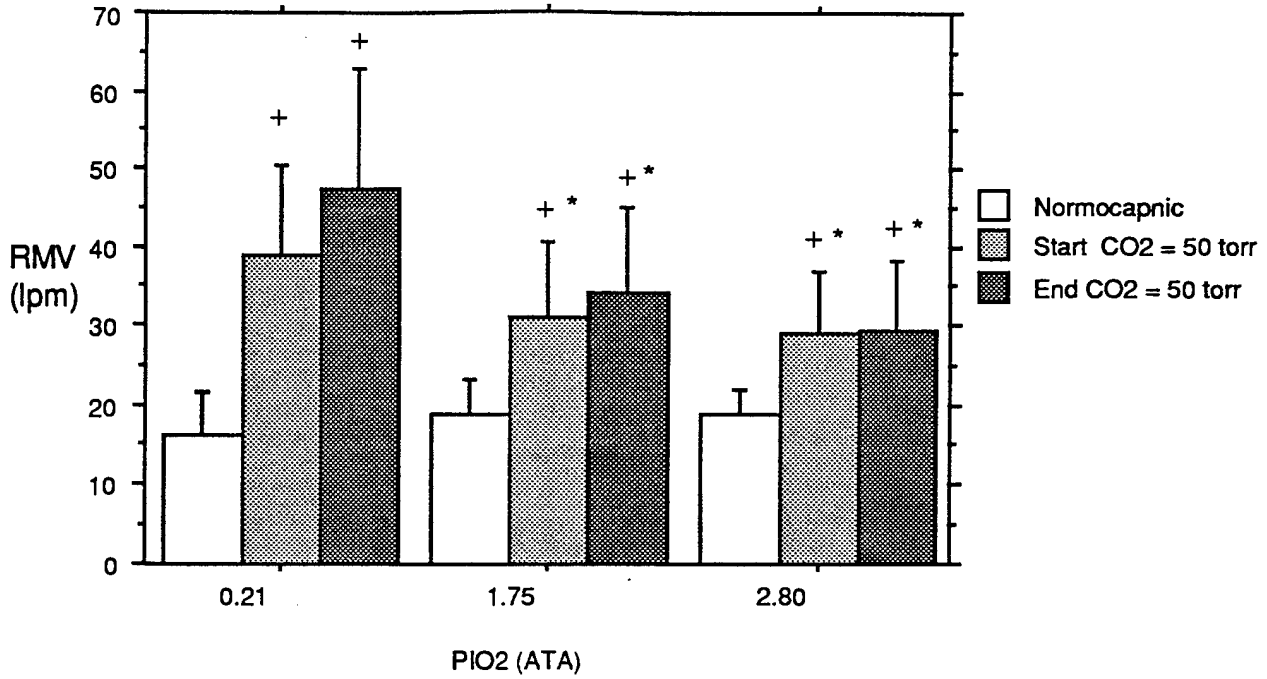

Figure 14: Mean ventilation changes: Mean ± SD values recorded during the last two minutes prior to rebreathing (normocapnia) and during the first two and last two minutes of the 10 minute rebreathing period (hypercapnia) at each PIO_2. n = 9

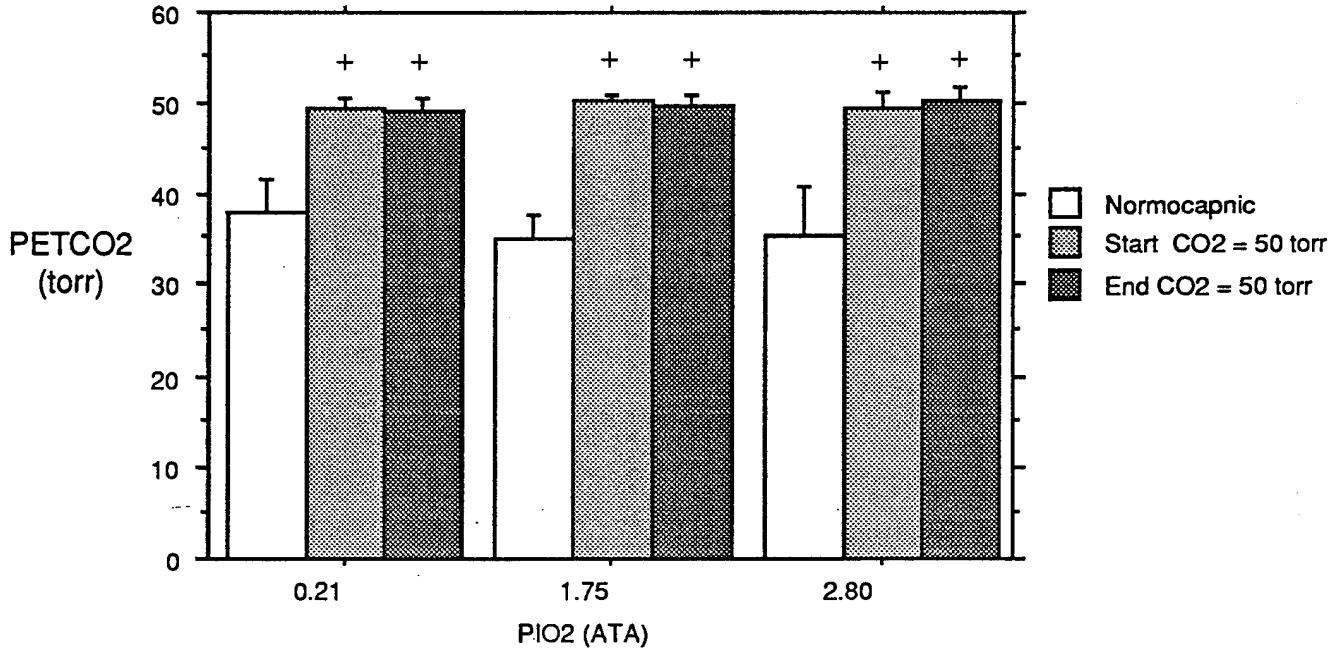

Figure 15: Mean PET_{CO2} changes: Mean ± SD values recorded during the last two minutes prior to rebreathing (normocapnia) and during the first two and last two minutes of the 10 minute rebreathing period (hypercapnia) at each PIO_2. n = 9

3.3.2 Cerebral Oxygenation

Rebreathing CO_2 to 50 torr $P_{ET}CO_2$ increased $tHbO_2$, tBV, and oxidized cyt. a,a_3 while tHb decreased while breathing at that level of CO_2 for 10 minutes further enhanced these changes as shown in a typical experiment in Figure 16.

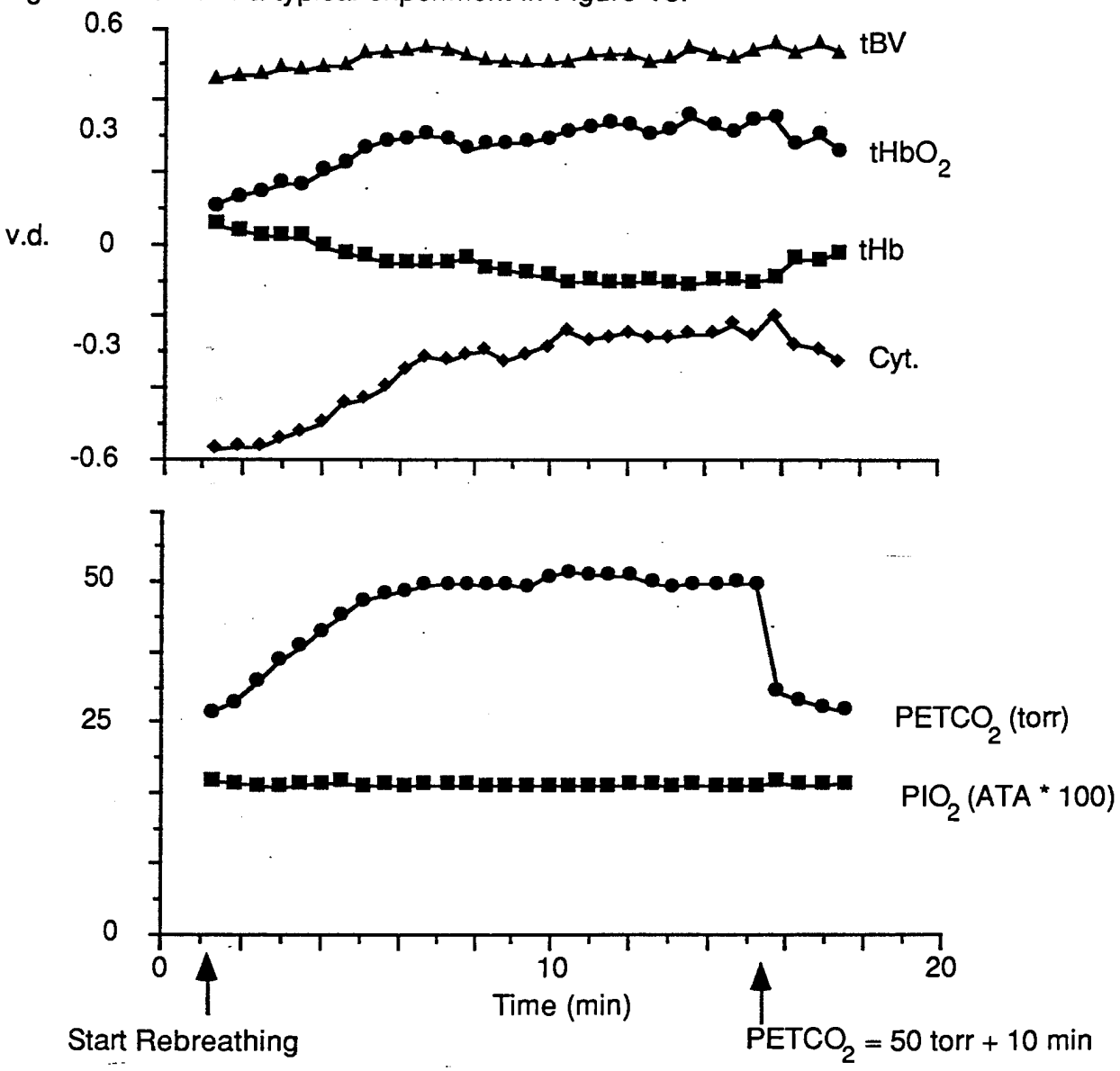

Figure 16: Typical CO_2 duration experiment. Cerebral oxygenation measurements - brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 and PIO_2 and $P_{ET}CO_2$ are shown for an experiment with normoxia and dry rest.

Mean cerebral oxygenation data are expressed as changes in NIR signals from control which was taken as the level after 5 minutes of normoxic, normocapnic, normobaric breathing. In Phase 3, changes from baseline were calculated after 5 minutes of breathing gas mixtures with no inspired CO_2 at each P_{IO_2} setpoint, when $P_{ET}CO_2$ reached 50 torr

during CO_2 rebreathing, and after 10 minutes of CO_2 rebreathing at that level. Mean data from the 9 completed experiments are shown in Figure 17.

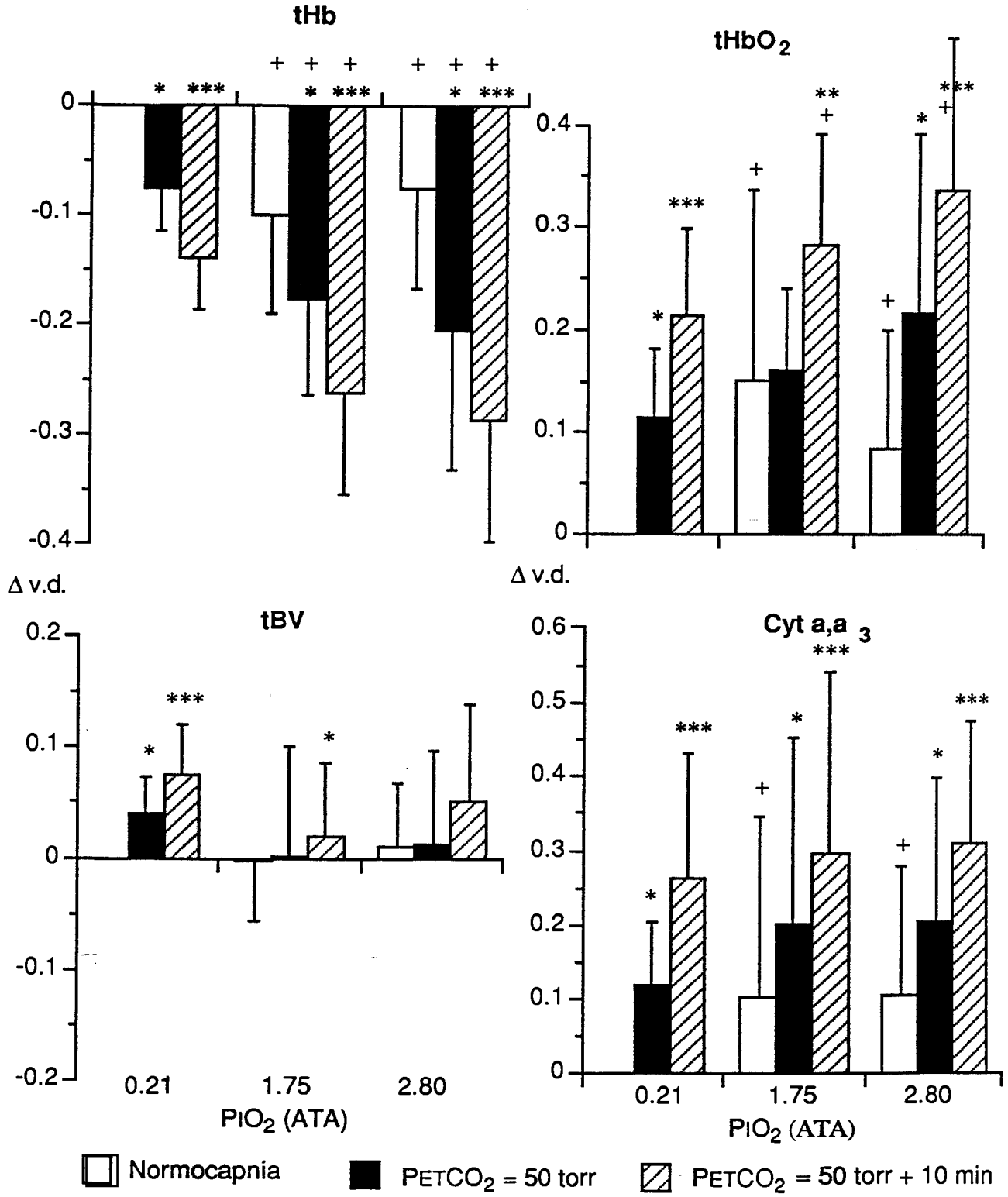

Figure 17: Mean cerebral oxygenation changes: . Changes in variations in density (Δvd) from control in brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 at three levels of CO_2 * Sig. diff. from normocapnia, $p < 0.05$. ** Sig. diff. from $PET_{CO_2} = 50$ torr, $p < 0.05$. *** Sig. diff. from both previous conditions, $p < 0.05$. + Sig. diff. from 0.21 ATA PIO_2, $p < 0.05$.

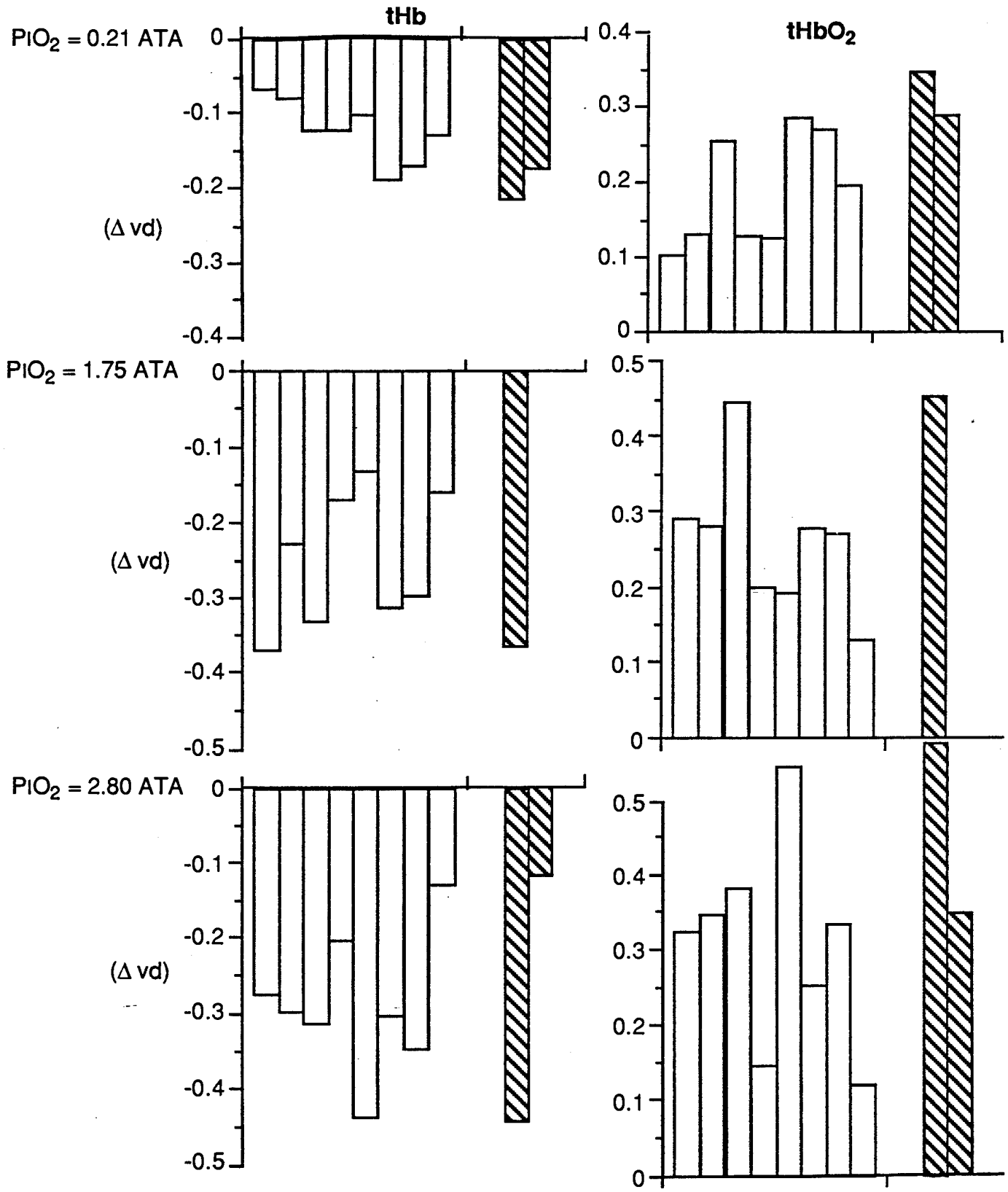

Figure 18 : Individual cerebral oxygenation changes. Changes in variations in density (Δvd) from control in brain tHb and $tHbO_2$ at three PIO_2's for PET_{CO_2} = 50 torr + 10 min. 1.75 ATA values are missing due to the convulsion.

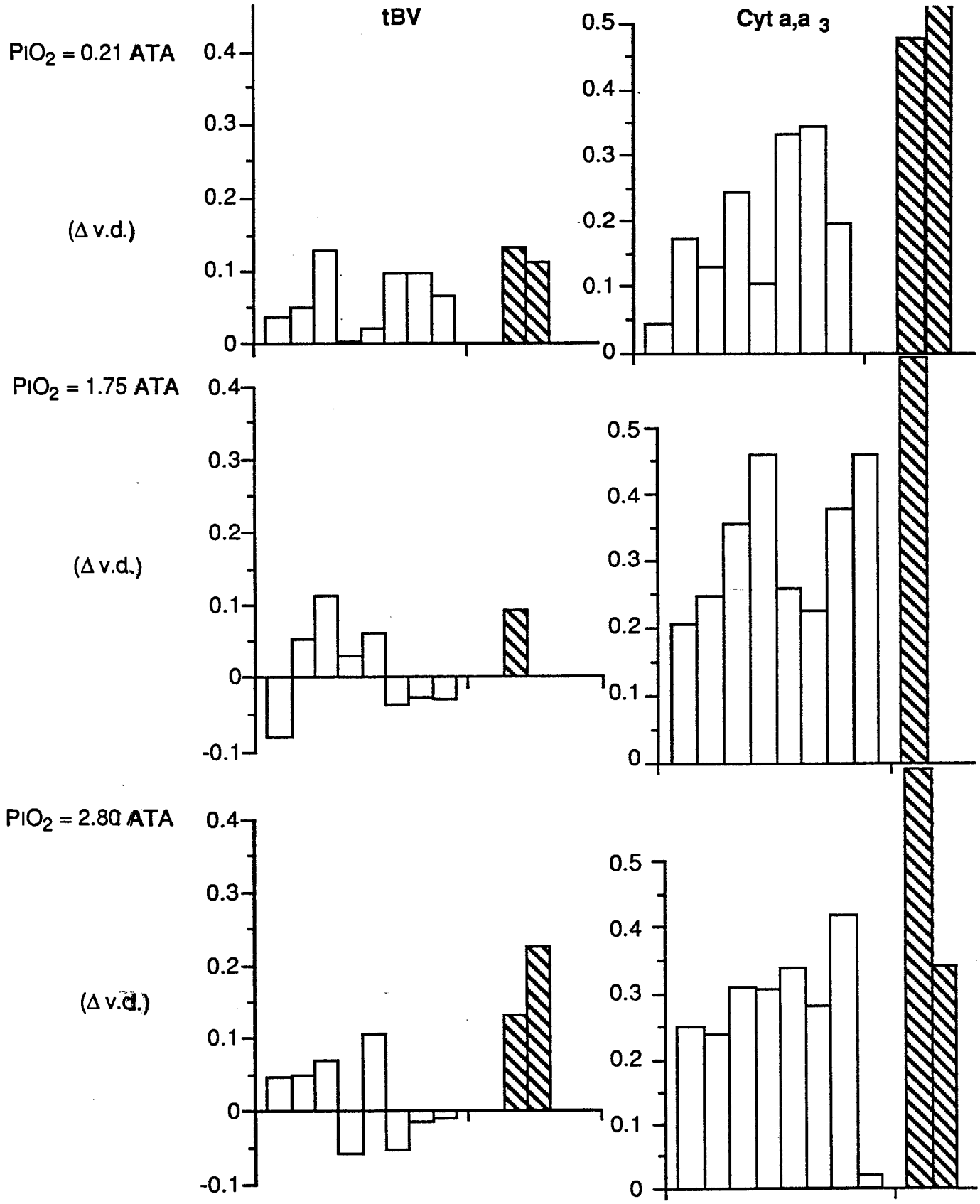

Figure 19: Individual cerebral oxygenation changes. Changes in variations in density (Δvd) from control in brain tBV and oxidized Cyt a,a_3 at three PIO_2's for PET_{CO_2} = 50 torr + 10 min. 1.75 ATA values are missing due to the convulsion.

Figures 18 and 19 show changes in NIR signals between control and PET_{CO_2} = 50 torr + 10 min. for all 10 subjects. Striped bars represent the subjects with mild symptoms or convulsion at 2.80 ATA PI_{O_2}. The subject with the convulsion appears as the far right bar.

Hyperoxia decreased tHb, increased $tHbO_2$, and cyt. a,a_3 . tBV was not significantly altered by hyperoxia though there was a trend toward vasoconstriction. The changes at 1.75 ATA PI_{O_2} were not significantly different from those at 2.80 ATA PI_{O_2} .

Rebreathing to PET_{CO_2} = 50 torr decreased tHb, increased $tHbO_2$, and increased oxidation of cyt. a,a_3 at each PI_{O_2}. Rebreathing at PET_{CO_2} = 50 torr for 10 min significantly enhanced these changes at each PI_{O_2}. tBV increased with a rise in PET_{CO_2} to 50 torr and increased further with rebreathing at this level for 10 min with a PI_{O_2} of 0.21 ATA but not during hyperoxia with the exception of the PET_{CO_2} = 50 torr + 10 min exposure at PI_{O_2} = 1.75 ATA.

The subject with mild symptoms had the largest cerebral oxygenation changes except for the subject who convulsed who had the largest cytochrome oxidation at a PI_{O_2} of 0.21 ATA and PET_{CO_2} = 50 torr + 10 min.

3.3.3 Symptoms

Symptoms were reported during the 2.80 ATA PI_{O_2} exposure only. One subject convulsed and one subject reported symptoms consistent with mild CNS oxygen toxicity. The subject who convulsed did not report symptoms prior to the convulsion. The subject reporting mild symptoms described them as nausea and light-headedness.

The convulsion took place 1 minute after the 10 minute CO_2 rebreathing period. The subject reporting mild symptoms experienced them during the last 2 minutes of the 10 minute CO_2 rebreathing period at 2.80 ATA PI_{O_2}. Symptoms abated upon the decrease of inspired CO_2.

The convulsion was a grand mal seizure. The subject dropped his mouthpiece and yelled out loudly as the seizure began 1 minute after the CO_2 level was decreased. The PI_{O_2} was 2.83 ATA, the PET_{CO_2} was 35 torr, and the inspired CO_2 was < 0.5% just prior to the convulsion. The subject shook vigorously for approximately 4 minutes and was unconscious

for another 4 minutes. Within 15 minutes, the subject was responsive and coherent at which time the chamber was decompressed. The subject was fully recovered after one hour except for extreme fatigue.

3.4 Phase 4 - Oxygen Duration Studies

Eleven subjects, 10 male and 1 female, mean (±SD) age = 34.2 ± 6.6 were tested in 11 sets of experiments. Each subject completed an experiment at 35 fsw (1.75 ATA PIO_2 for 40 min) and another at 77 fsw (2.80 ATA PIO_2 for 20 min) on a different day. Experiments were at least 24 hrs apart and at most 10 days apart.

3.4.1 Ventilatory Parameters

Ventilation did not change significantly with increasing PIO_2 or duration of exposure. $PETCO_2$ decreased with hyperoxia and decreased further after 40 min at 1.75 ATA PIO_2 (Figure 20).

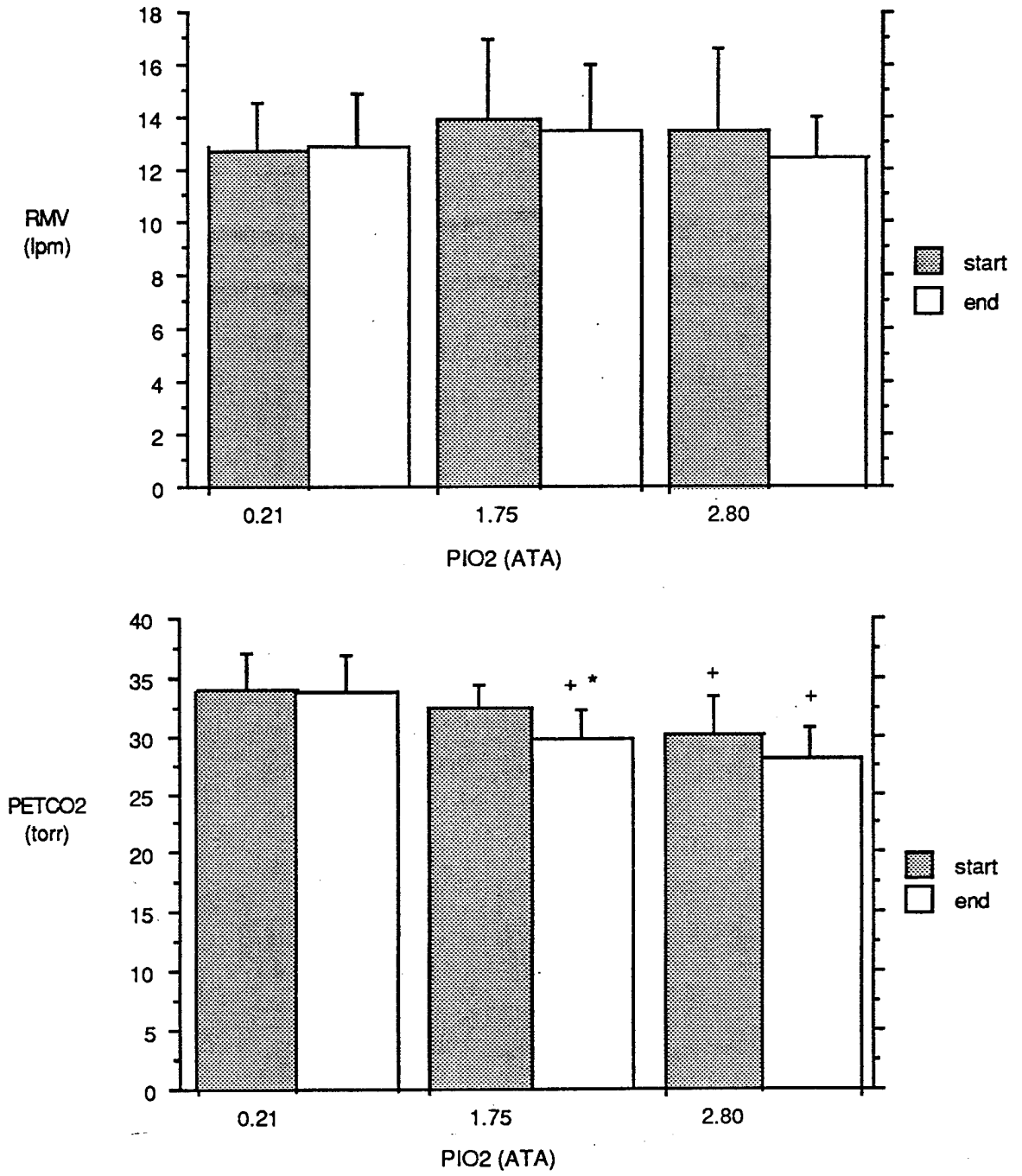

Figure 20 : Mean ventilation and PET_{CO2} changes dry at rest. Mean ± SD values recorded during the first two and last two minutes of the 5 min (PIO2 = 0.21), 40 min (PIO2 = 1.75) and 20 min (PIO2 = 2.80) breathing periods. n = 11 * Sig. diff. from start, $p < 0.05$. + Sig. diff. from 0.21 ATA PIO_2 , $p < 0.05$.

3.4.2 Cerebral Oxygenation

Mean cerebral oxygenation data are expressed as changes in NIR signals from control which was taken after 5 minutes of normoxic, normocapnic, normobaric breathing. Changes

from baseline were calculated after breathing gas for 10 minutes at 0.21 ATA P_{IO_2}, 40 minutes at 1.75 ATA P_{IO_2}, and 20 minutes at 2.80 ATA P_{IO_2}. Mean data from the 11 completed experiments are shown in Figure 21. Data from 0.21 ATA P_{IO_2} exposures are pooled because all conditions were the same.

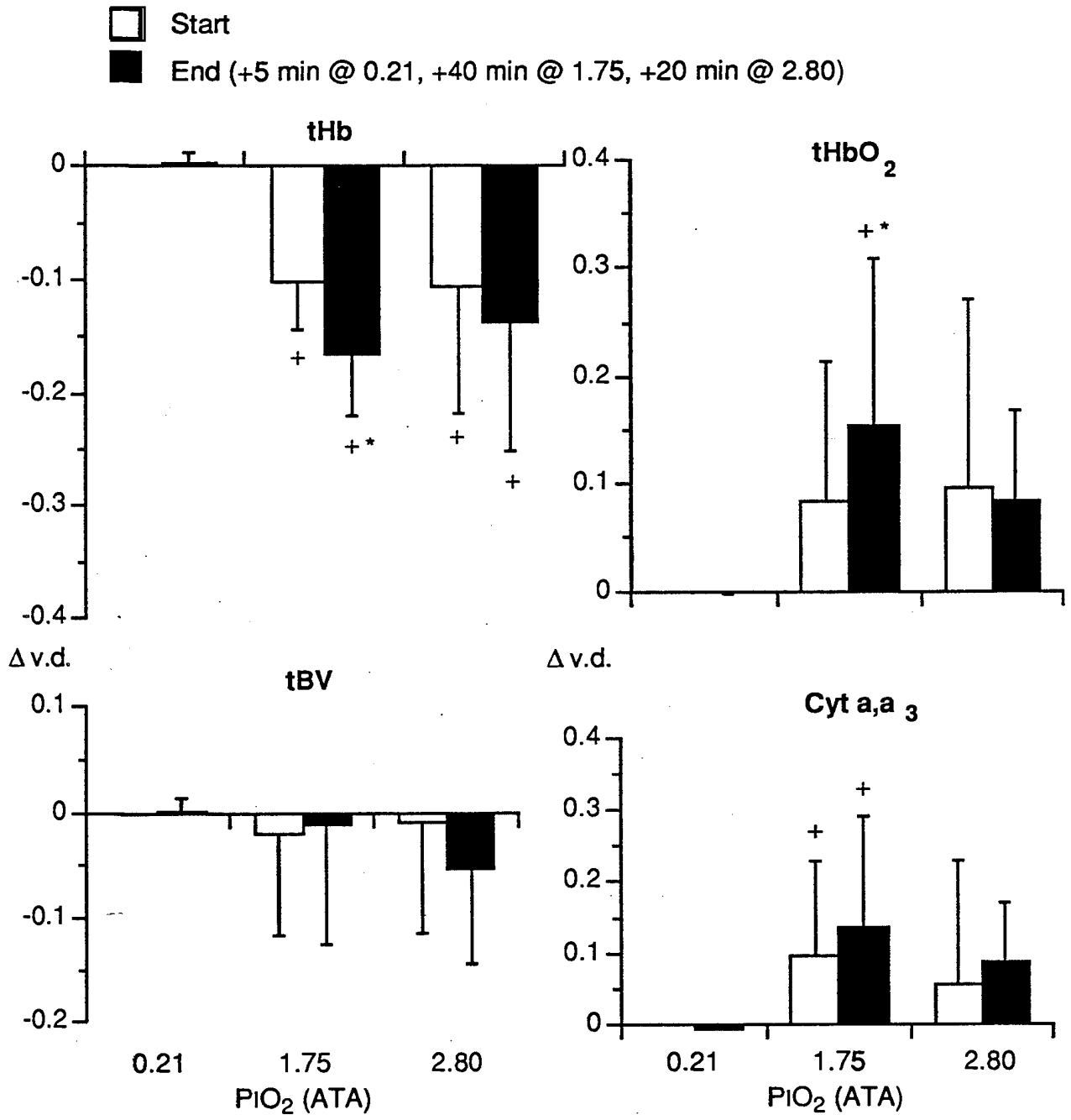

Figure 21: Mean cerebral oxygenation changes. Changes in variations in density (Δvd) from control in brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 at the start and end of the 5 min (PIO2 = 0.21), 40 min (PIO2 = 1.75) and 20 min (PIO2 = 2.80) breathing periods. * Sig. diff. from start, $p < 0.05$. + Sig. diff. from 0.21 ATA P_{IO_2}, $p < 0.05$.

Hyperoxia decreased tHb, increased $tHbO_2$, and cyt. a,a_3 but not significantly at 2.80 ATA P_{IO_2} for $tHbO_2$ and cyt. a,a_3. tBV was not significantly altered by hyperoxia though there was a trend toward vasoconstriction. The changes at 1.75 ATA P_{IO_2} were not significantly different from those at 2.80 ATA P_{IO_2} although there is a decreasing trend suggesting autoregulation.

Duration caused significant further decrease in tHb and increase in $tHbO_2$ only at 1.75 ATA P_{IO_2} after 40 min. No significant changes were measured after 20 min at 2.80 ATA P_{IO_2}.

3.4.3 Symptoms

No symptoms were reported during either the 1.75 ATA P_{IO_2} or the 2.80 ATA P_{IO_2} exposure.

3.5 Phase 5 - Head-out Immersion and Phase 6 - Total Immersion

In Phase 5, 6 subjects, 5 male and 1 female, mean (±SD) age = 29.5 (± 4.4) were tested in 6 sets of experiments. Each subject completed an experiment at 35 fsw (1.75 ATA PIO2 for 40 min) and another at 77 fsw (2.80 ATA PIO2 for 20 min) on a different day. Experiments were at least 24 hrs apart and at most 16 days apart. In Phase 5, subjects were immersed to the neck in thermoneutral water, 34° C ± 1°C.

In Phase 6, 6 subjects, 5 male and 1 female, mean (±SD) age = 35.3 (± 8.2) were tested in 6 sets of experiments. Each subject completed an experiment at 35 fsw (1.75 ATA PI_{O_2} for 40 min) and another at 77 fsw (2.80 ATA PI_{O_2} for 20 min) on a different day. Experiments were at least 24 hrs apart and at most 4 days apart except in two cases where experiments were repeated 3 months later due to loss of data during the initial experiments. In Phase 6, subjects were fully immersed in thermoneutral water, 34° C ± 1°C.

3.5.1 Ventilatory Parameters

Figure 22 shows mean ventilatory data for Phases 5 and 6. Ventilation did not change significantly with increasing PI_{O_2}. PET_{CO_2} decreased with increasing hyperoxia for the duration of the head out experiments and by the end of the total immersion experiments. Ventilation did not change significantly with the duration of the exposure. PET_{CO_2} decreased after the 20 min duration at 2.80 ATA PI_{O_2}.

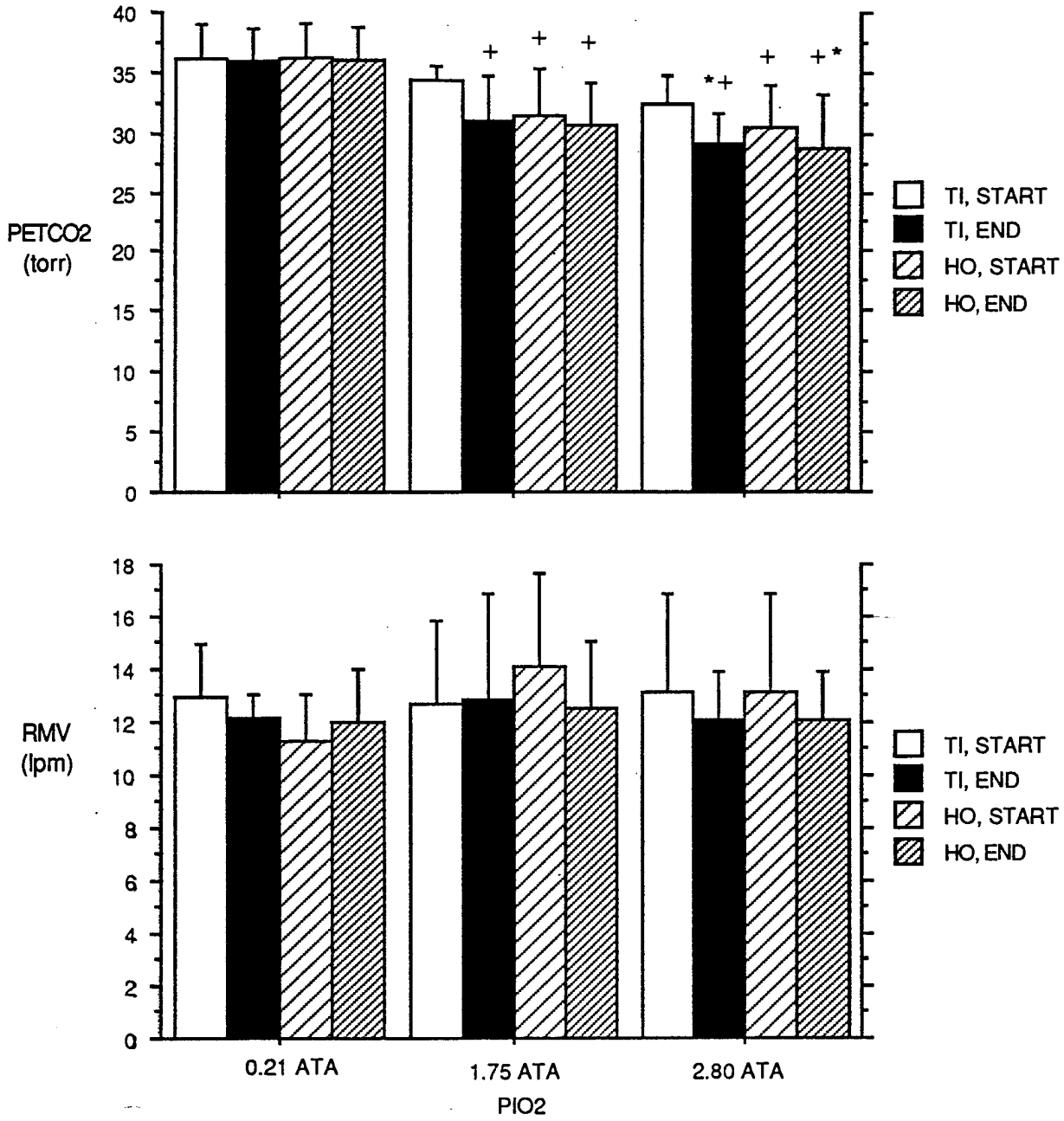

Figure 22: Mean ventilation and PET_{CO_2} during head out and total immersion Mean ± SD values recorded during the first two and last two minutes of the 10 min (PIO2 = 0.21), 40 min (PIO2 = 1.75) and 20 min (PIO2 = 2.80) breathing periods. n = 11 * Sig. diff. from start, $p < 0.05$. + Sig. diff. from 0.21 ATA PIO_2 , $p < 0.05$.

3.5.2 Cerebral Oxygenation

Mean cerebral oxygenation data are expressed as changes in NIR signals from baseline which was recorded after 5 minutes of normobaric, normocapnic, normoxia while immersed

to the neck (Phase 5) or fully immersed (Phase 6). In Phases 5 and 6, changes from baseline were calculated after 10 minutes at 0.21 ATA P_{IO_2}, at the start and end of a 40 minute period at 1.75 ATA P_{IO_2}, and at the start and end of a 20 minute period at 2.80 ATA P_{IO_2}. Mean data from 12 sets of experiments (6 in Phase 5 and 6 in Phase 6) are shown in Figure 23.

tHb decreased and $tHbO_2$ increased with increasing P_{IO_2} for both head-out and total immersion. Oxidation of cytochrome a,a_3 tended to increase with increasing P_{IO_2} but was significantly higher only at 2.80 ATA P_{IO_2} after 20 minutes of total immersion. tBV tended to decrease with increasing P_{IO_2} and was significantly smaller at 2.80 ATA P_{IO_2} after 20 min of immersion, head-out and total. No significant differences were observed between head-out immersion and total immersion.

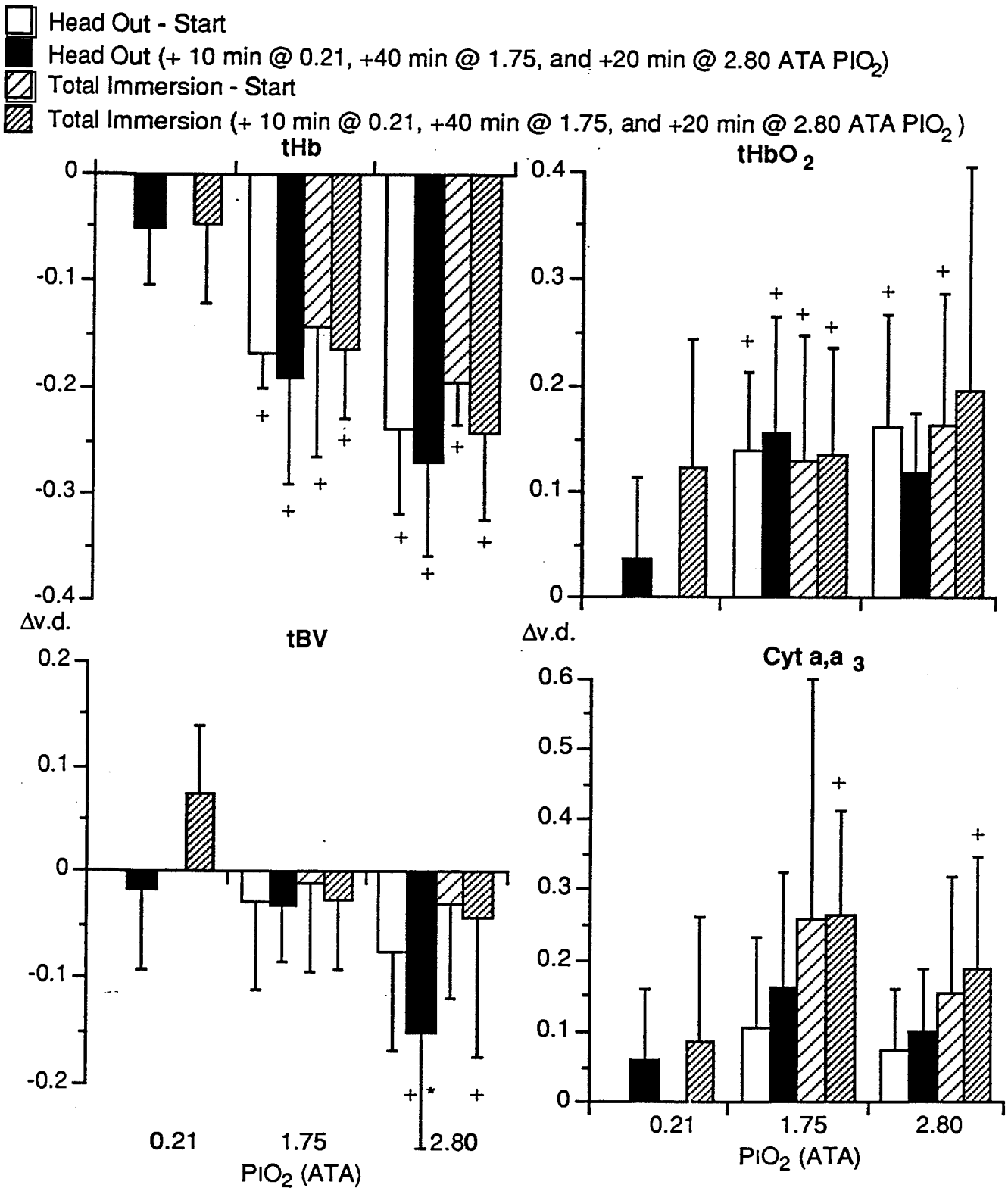

Figure 23: Effect of duration on mean cerebral oxygenation changes during head out and total immersion. Changes in variations in density (Δvd) from control in brain tHb, tHbO2, tBV, and oxidized Cyt a,a3 at the start and end of the 10 min (PIO2 = 0.21), 40 min (PIO2 = 1.75) and 20 min (PIO2 = 2.80) breathing periods for head out and total immersion. * Sig. diff. from start, $p < 0.05$. + Sig. diff. from 0.21 ATA PIO2 , $p < 0.05$.

3.5.3 Symptoms

No symptoms were reported during either the 1.75 ATA PIO_2 or the 2.80 ATA PIO_2 exposure in Phase 5 or 6.

3.6 Phase 7 - Facial Immersion and Phase 8 - Static Lung Load and the Dive Reflex

In Phase 7, 12 subjects, 10 male and 2 female, mean (±SD) age = 33.3 (± 6.1) were tested in 10 experiments. The effects of facial immersion were tested by immersing subjects to the neck for 10 minutes and then allowing the water level to rise over the face until fully immersed for 10 min in thermoneutral water, 35° C ± 1°C. Experiments were done at the surface and then at 77 fsw (2.80 ATA PIO_2). One experiment resulted in a loss of data and another was cut short by the subject. Mean data from the ten complete experiments are reported. Six subjects completed 10 minute recovery periods in the head-out condition following total immersion.

In Phase 8, 6 subjects, 5 male and 1 female, mean (±SD) age = 33.2 (± 6.5) were tested in 6 experiments. Subjects were immersed to the neck, then fully immersed, returned to the head out condition, and fully immersed again for 10 min durations in thermoneutral water, 35° C ± 1°C. To test the effect of static lung load on cerebral oxygenation, subjects breathed air on the surface, 0.21 ATA PIO_2, from a modified Mark 15 UBA which had a positive static lung load (+10 cm H_2O). In previous phases (6 and 7), the closed-circuit rebreathing system had a negative static lung load (-30 cm H_2O). Comparisons of measurements taken at the surface in Phase 7 and measurements taken in Phase 8 are compared to investigate the role of static lung load in cerebral oxygenation. Ventilatory measurements were not taken during breathing on the modified Mark 15 UBA. One minute breath holds were performed in the middle of the first head-out exposure and the last total immersion exposure to simulate

conditions provoking the dive reflex in mammals, apneic facial immersion. The effect of the dive reflex on cerebral oxygenation was investigated. Heart rate was measured in both Phases 7 and 8. Bradycardia is a measurable characteristic of the dive reflex.

3.6.1 Ventilatory Parameters and Heart Rate

Figure 24 shows the mean ventilatory and heart rate data from Phase 7. Ventilation did not change significantly with increased P_{IO_2}. $P_{ET_{CO_2}}$ decreased at 2.80 ATA P_{IO_2} compared to 0.21 ATA P_{IO_2} for each immersion condition. Heart rate decreased at 2.80 ATA P_{IO_2} in both head out and total immersion (heart rate data was not obtained during recovery). In Phase 8, a single P_{IO_2} was tested (0.21 ATA) and ventilation was not measured because a Mark 15 UBA was used.

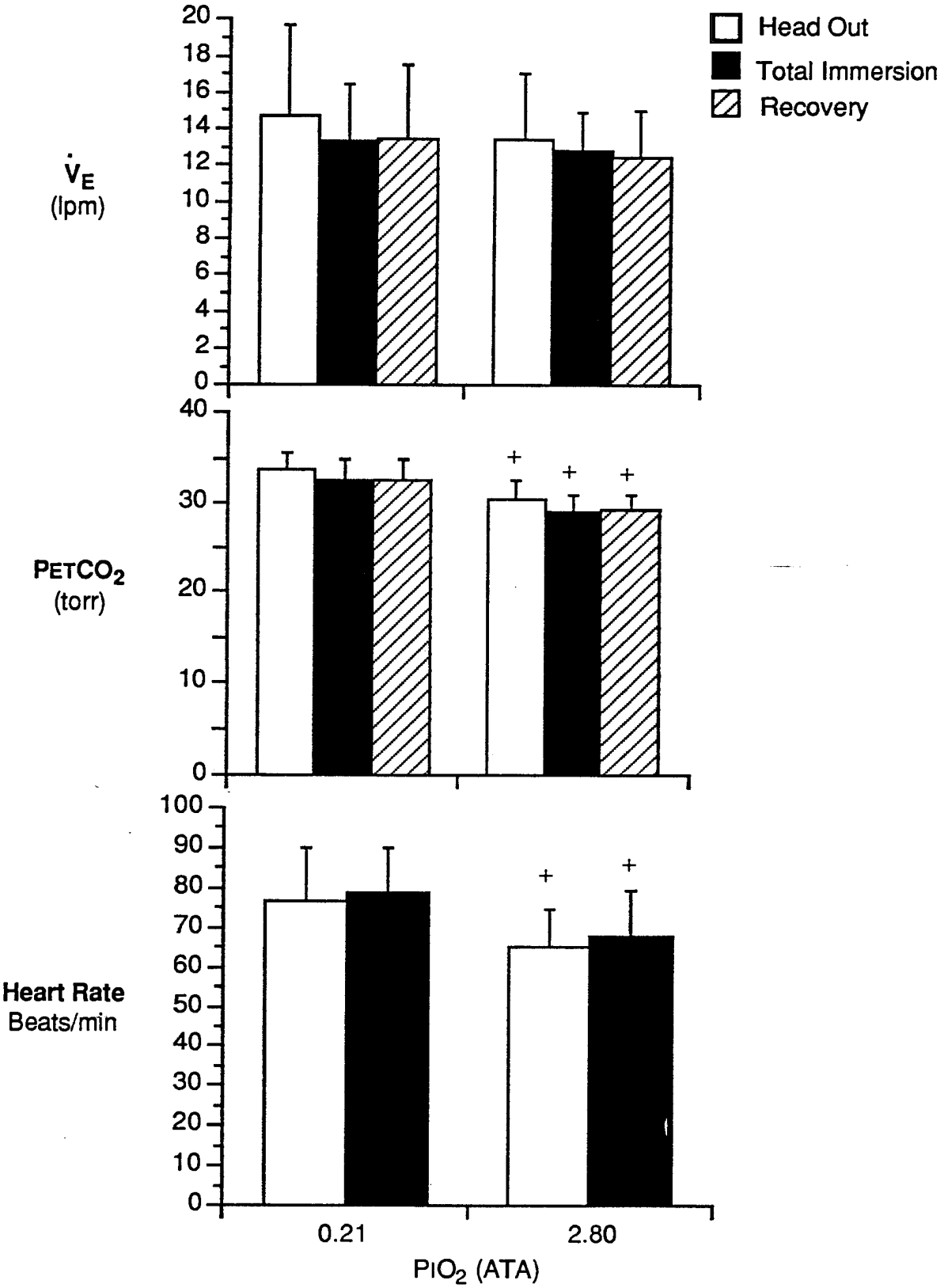

Figure 24: Mean ventilation, PET_{CO_2}, and heart rate during immersion with negative static lung loading. • Sig. diff. from head out, $p < 0.05$. + Sig. diff. from 0.21 ATA PIO_2, $p < 0.05$.

In Phase 7 no significant differences in ventilation, $P_{ET}CO_2$, or heart rate were found due to total immersion compared to head-out immersion (Figure 24). In Phase 8, breath hold significantly decreased heart rate during both head out and total immersion (Figure 25).

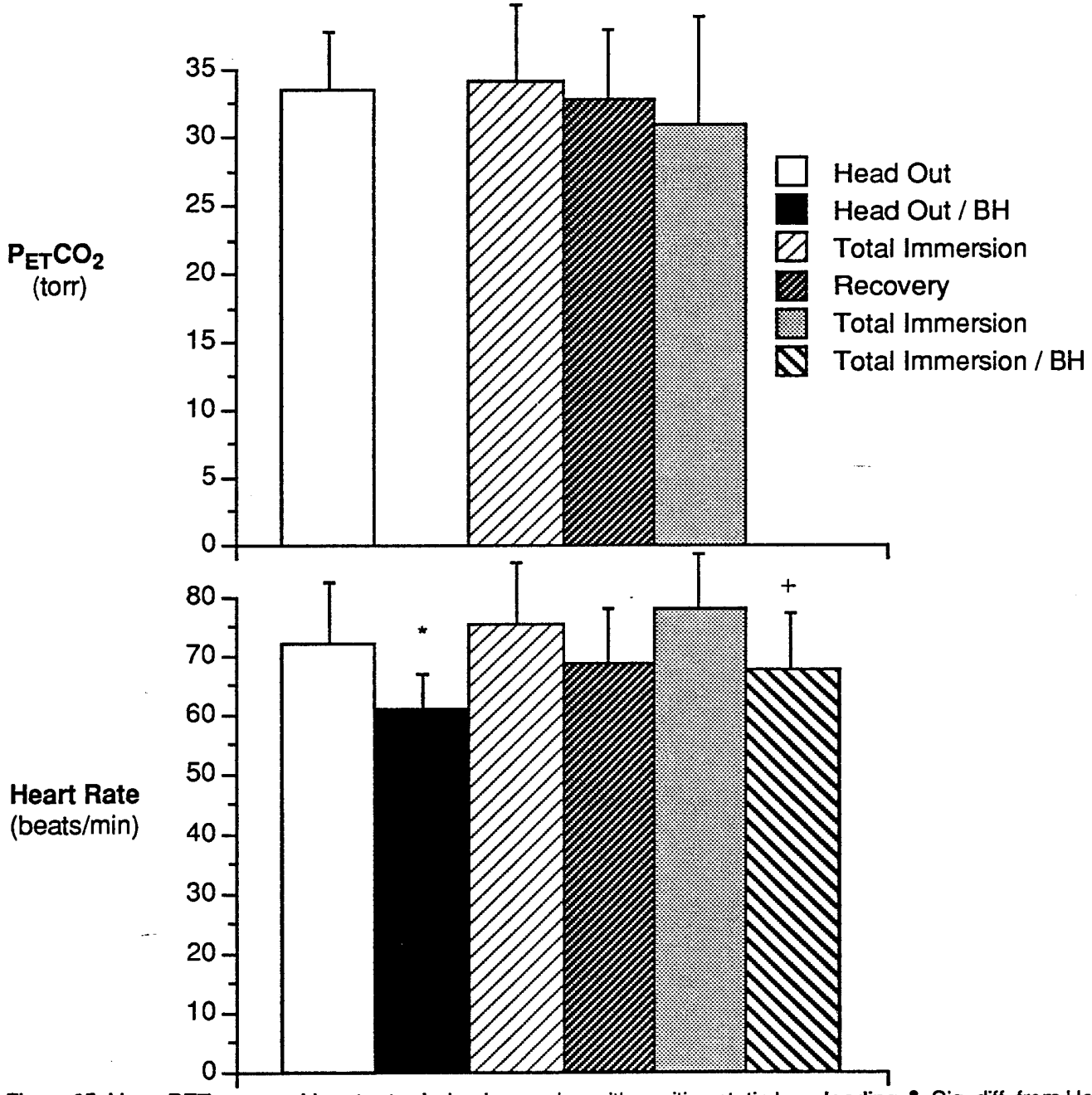

Figure 25: Mean PET_{CO_2}, and heart rate during immersion with positive static lung loading. • Sig. diff. from Head Out immersion, $p < 0.05$. + Sig. diff. from Total immersion, $p < 0.05$.

3.6.3 Cerebral Oxygenation

In Phase 7, changes from baseline were calculated after 10 min. at 0.21 ATA P_{IO_2} in the

head-out condition, 10 min. of total immersion, and 10 min. head-out again and again at 2.80 ATA P_{IO_2}. In a typical experiment (Figure 26), $tHbO_2$, tBV, and Cyt. a,a_3 increase with full immersion while tHb decreases. These changes are reversed when the water level is returned to the head out position.

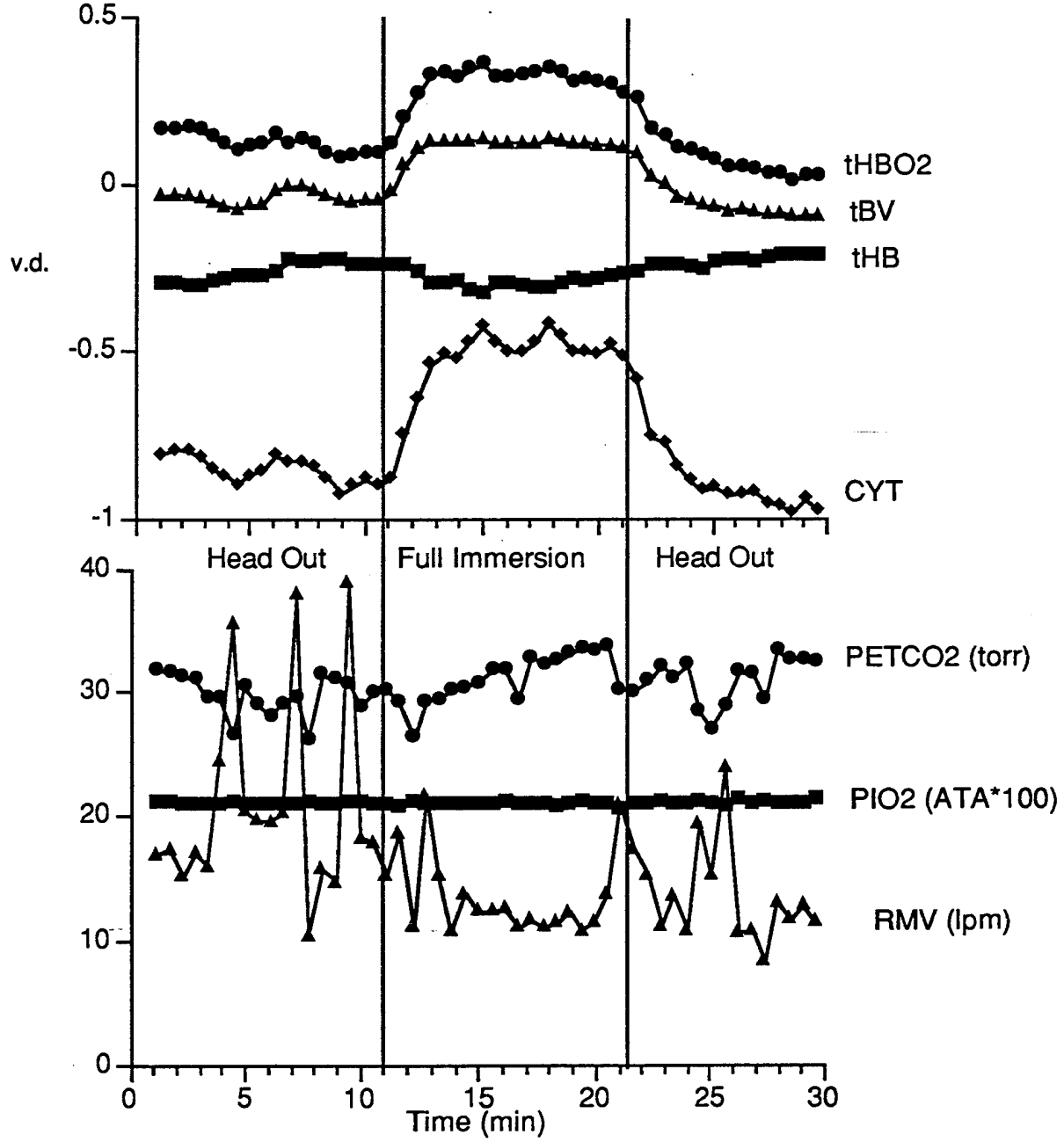

Figure 26: Typical head out / total immersion with negative static lung load experiment. Cerebral oxygenation measurements - brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 and ventilation, P_{IO_2}, and $P_{ET_{CO_2}}$ are shown for an experiment at the surface on air at rest.

Mean cerebral oxygenation data are expressed as changes in NIR signals from baseline which was recorded after 5 minutes of normobaric, normocapnic, normoxia in the head out condition. Mean data from the 10 experiments are shown in Figure 27.

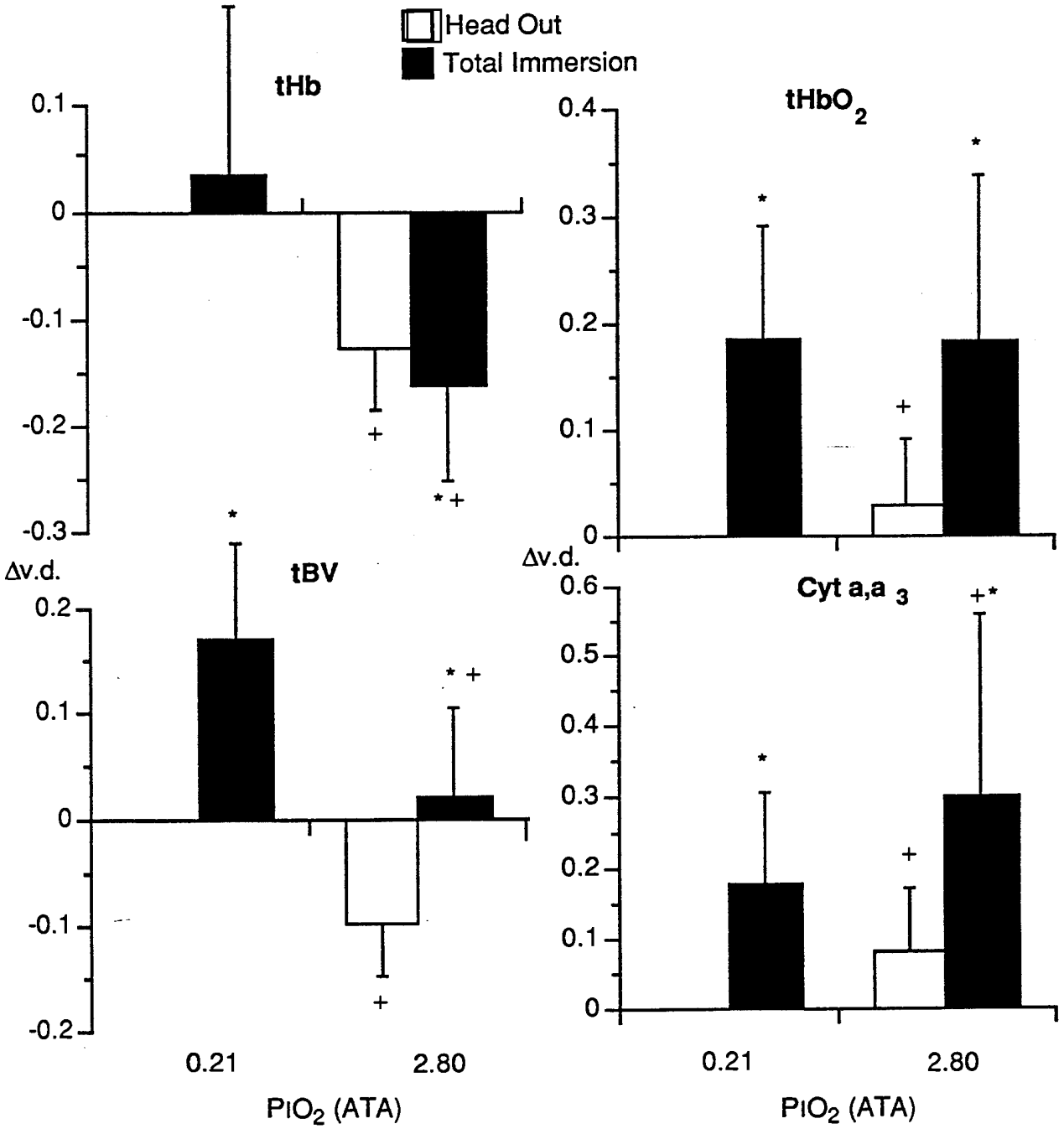

Figure 27: Mean cerebral oxygenation changes during immersion with negative static lung load. Changes in variations in density (Δvd) from control in brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 at the end of the 10 min head out and fully immersed at $PIO_2 = 0.21$ and 10 min head out and fully immersed at $PIO_2 = 2.80$. * Sig. diff. from head out, $p < 0.05$. + Sig. diff. from 0.21 ATA $p < 0.05$.

In Phase 8, changes from baseline were calculated after 10 minutes at 0.21 ATA P_{IO_2} in the head out condition, after a 1 minute breath hold half way through that period, after 10 minutes of total immersion, after 10 minutes of head out immersion, and after a 1 minute breath hold 5 minutes into another immersion period. All measurements were taken at 1 ATA with air breathing. A typical experiment is shown in Figure 28.

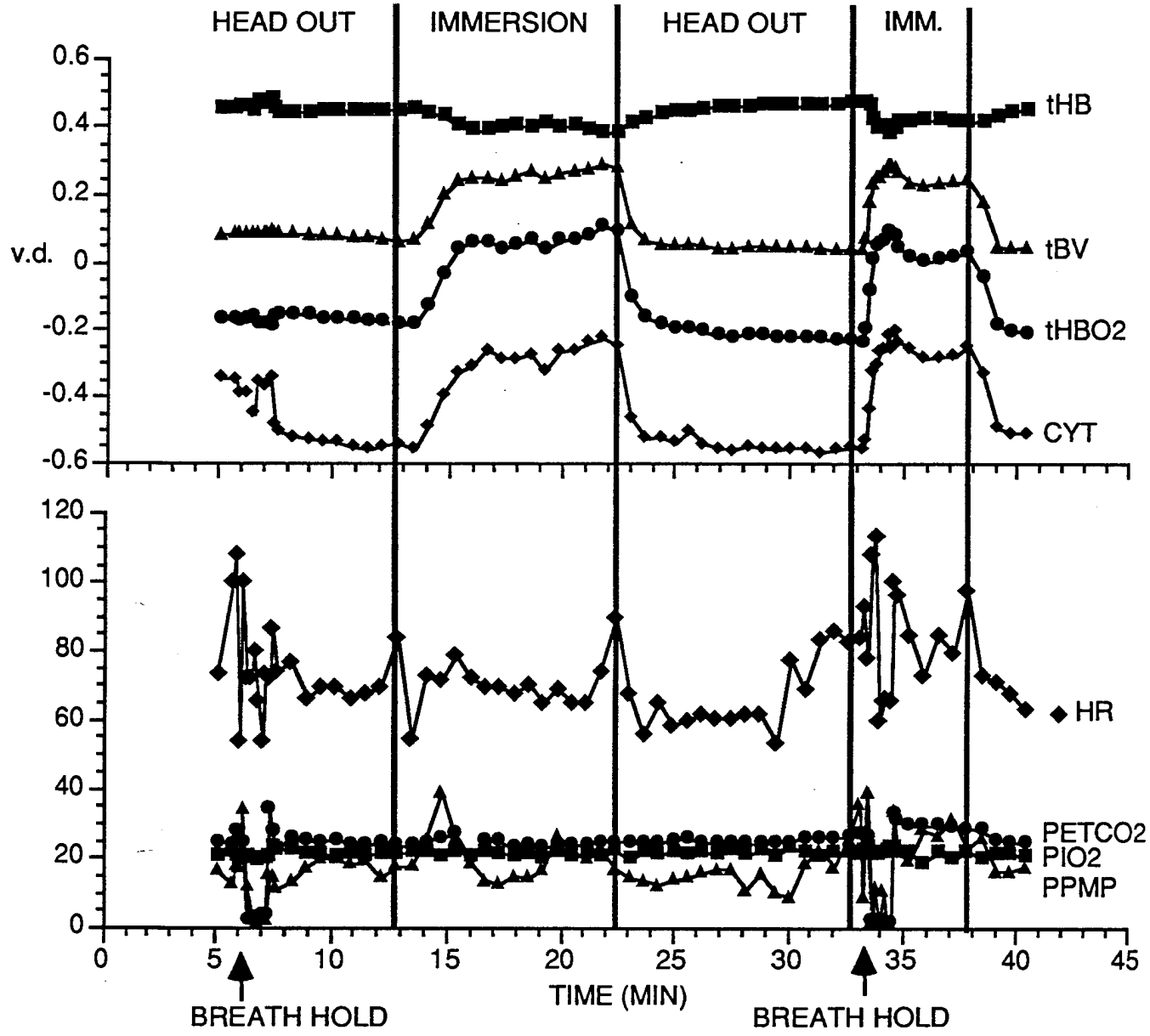

Figure 28: Typical head out / total immersion with positive static lung load experiment. Cerebral oxygenation measurements - brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 and heart rate, mouthpiece pressure, PIO_2, and PET_{CO_2} are shown for an experiment at the surface on air at rest.

Figure 29 shows the breath hold during total immersion on an expanded time scale. tHb and heart rate decrease while $tHbO_2$, tBV, and cyt. a,a_3 oxidation increase during the breath hold.

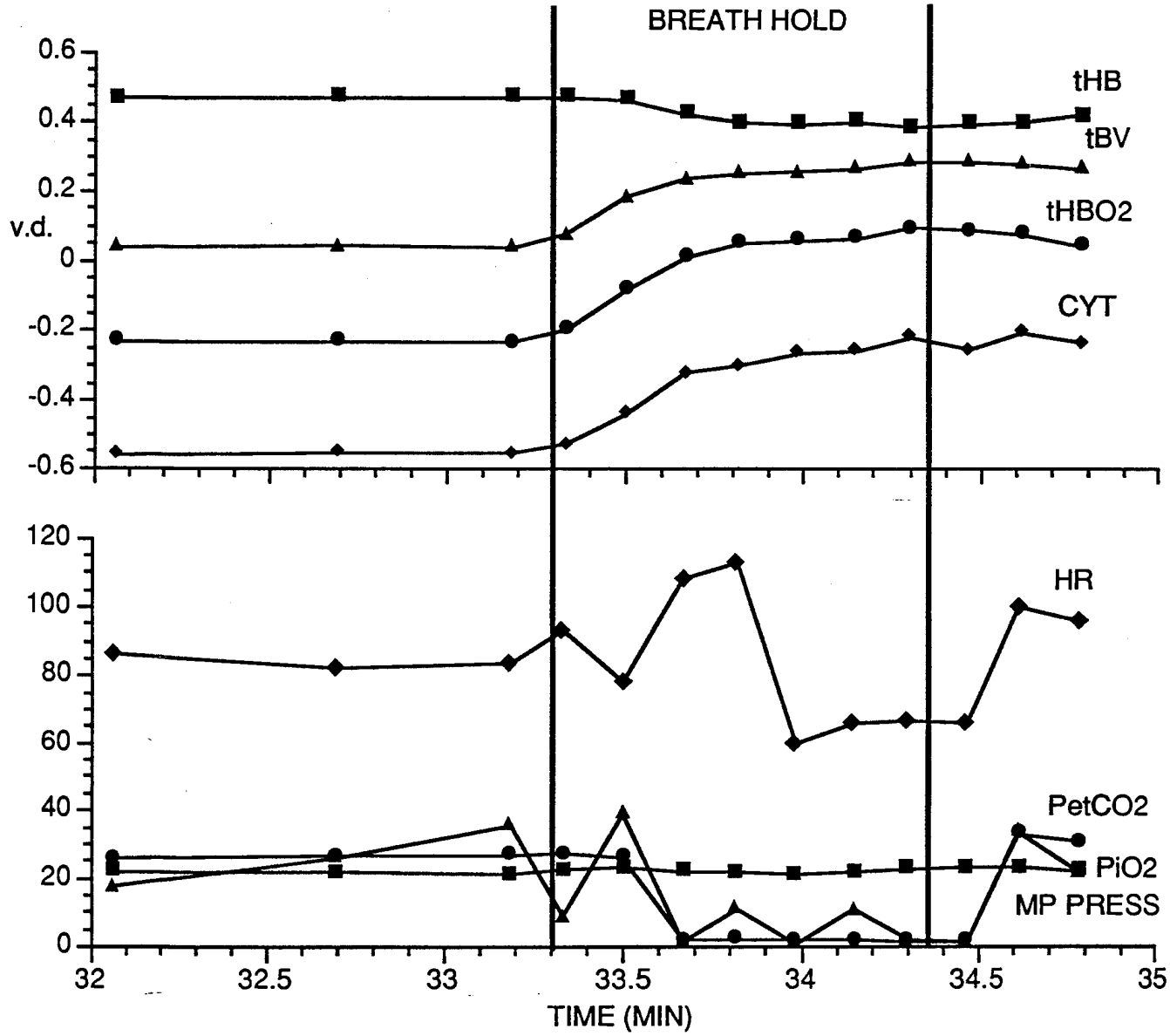

Figure 29: Breath hold during positive static lung load experiment. Cerebral oxygenation measurements - brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 and heart rate, mouthpiece pressure, PIO_2, and PET_{CO2} are shown for an experiment at the surface on air at rest.

Mean data from 6 experiments are shown in Figure 30.

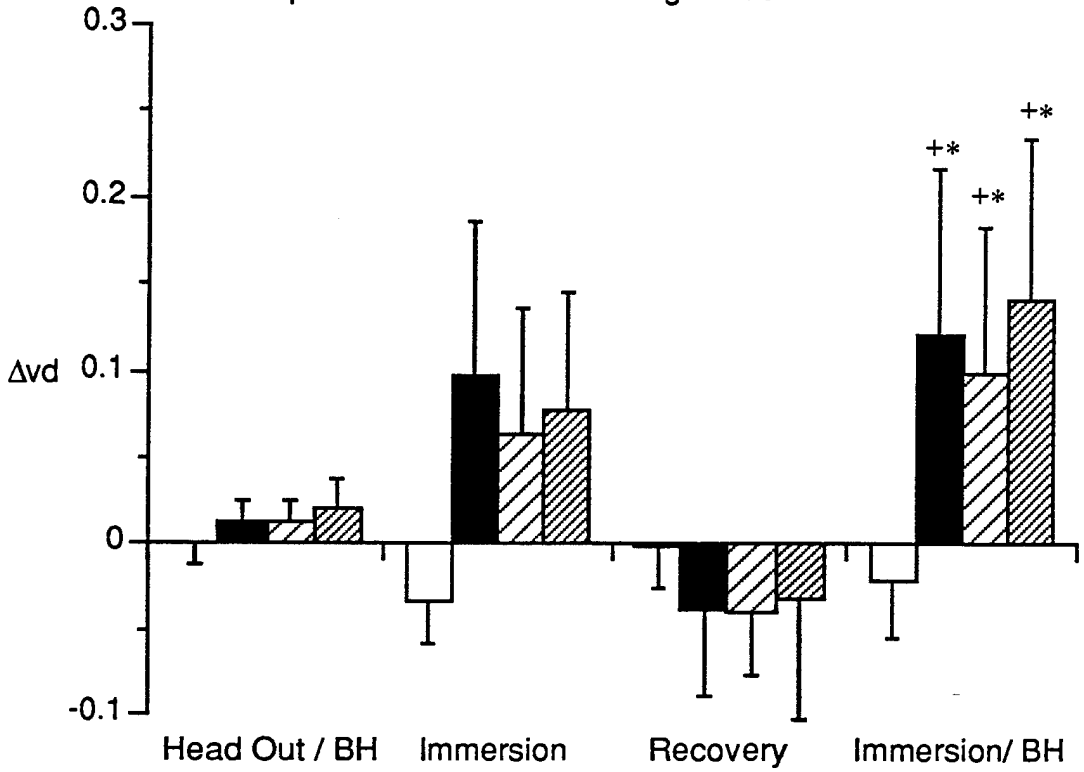

Figure 30: Mean cerebral oxygenation changes during immersion with a positive static lung load . Changes in variations in density (Δvd) from the head out control in brain tHb □, $tHbO_2$ ■, tBV ▨, and oxidized Cyt a,a_3 ▨ at $PIO_2 = 0.21$, for different immersion levels and after 1 minute breath holds. * Sig. diff. from immersion, $p < 0.05$. + Sig. diff. from Head Out / BH, $p < 0.05$.

Surface studies from Phases 7 and 8 are compared in Figure 31 to examine the effect of static lung load for n = 6 (recovery data was not obtained for 4 subjects in Phase 7). Significant decreases in $tHbO_2$, tBV, and cyt. a,a_3 oxidation were measured during recovery from total immersion in both the negative and positive static lung load experiments. A significant decrease in tHb was measured in the positive lung load experiments.

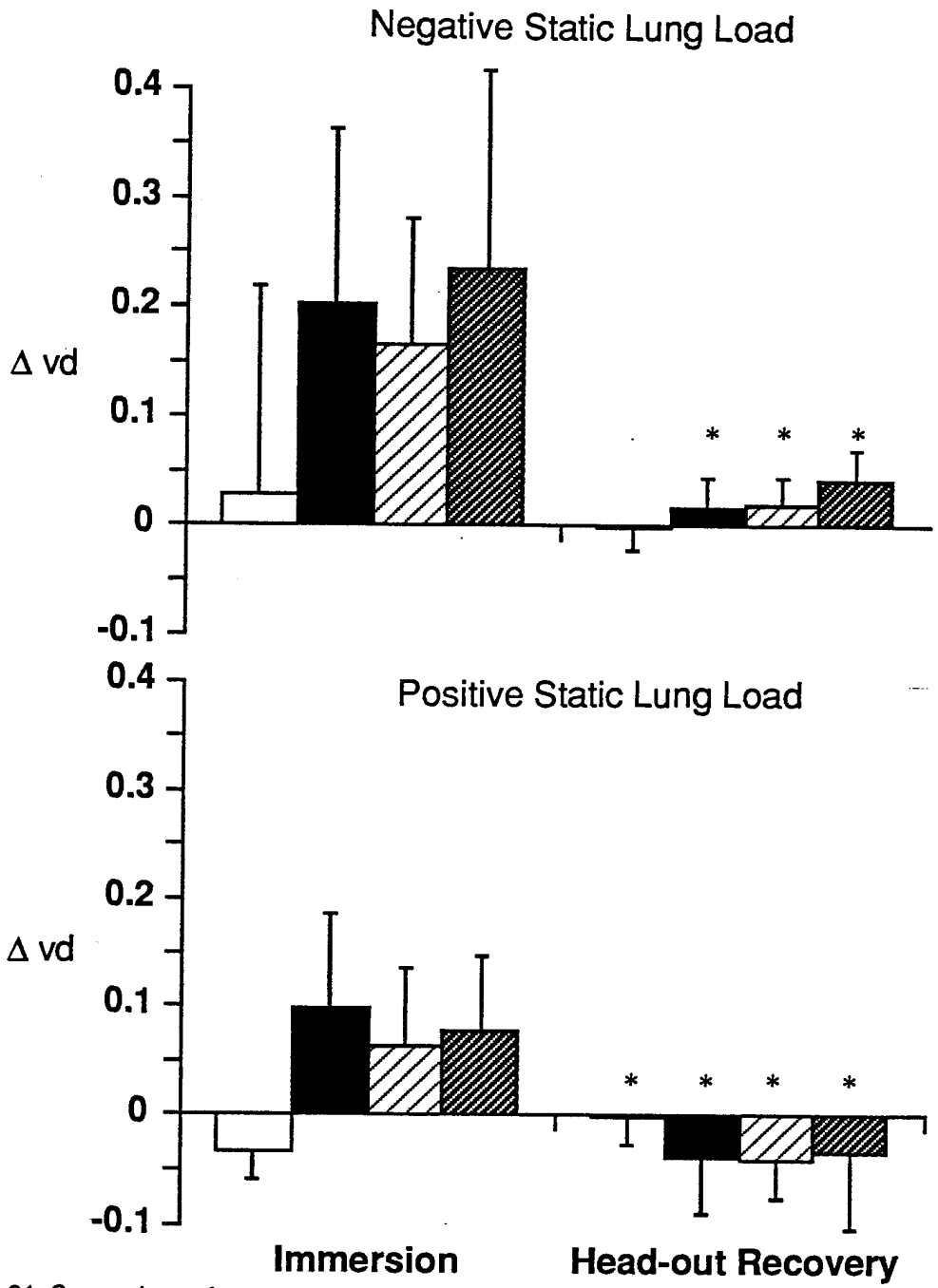

Figure 31: Comparison of mean cerebral oxygenation changes for negative and positive static lung loads . Changes in variations in density (Δvd) from head out control in brain tHb ☐, $tHbO_2$ ■, tBV ▨, and oxidized Cyt a,a_3 ▨ at the start and end of the 5 min (PIO2 = 0.21), 40 min (PIO2 = 1.75) and 20 min (PIO2 = 2.80) breathing periods. * Sig. diff. from immersion

3.6.3 Symptoms

One subject reported chills during the head out portion of the experiment and anxiety over breathing resistance during the total immersion at 2.80 ATA P_{IO_2} in Phase 7. The subject stopped the experiment at 12 min instead of proceeding to 20 min duration. The same subject reported disorientation the following morning which cleared by afternoon. No abnormalities were found during a subsequent neurological exam.

3.7 Phase 9 - Dry Work and Phase 10 - Totally Immersed Work

In Phase 9, 10 subjects, 8 male and 2 female, mean (±SD) age = 31.1 (± 7.0) were tested in 11 dry experiments at 0.21 and 1.75 ATA P_{IO_2}. One experiment was a repeat because the CO_2 canister broke through during the initial run. Work rate was determined by oxygen consumption and feedback to subjects as to RPM on the bicycle ergometer. In 4 of the experiments a 6 / 4 min. work rest cycle was employed to maintain an average oxygen consumption of 1.5 lpm.

In Phase 10, 11 subjects, 9 male and 2 female, mean (±SD) age = 31.5 (± 6.4) were tested in 12 experiments. Subjects were fully immersed in thermoneutral water, 30° C ± 1°C at 0.21 and 1.75 ATA P_{IO_2}. One experiment was aborted because the subject could not equalize their ears during compression. The same subjects' repeat experiment was aborted due to loss of data. One experiment was aborted because the subject stopped early due to apprehension while immersed at depth. Each aborted experiment involved a female subject leaving 9 experiments to be reported on with all male participants with a mean age of 33.1 (± 7.0).

3.7.1 Ventilatory Parameters and Heart Rate

Work rate was set by giving verbal feedback to subjects based on oxygen consumption determinations. A target of 1.5 lpm was used during working experiments. In dry studies, a 6 / 4 min work rest cycle was used to achieve an average oxygen consumption during the 40 min work period at 1.75 ATA P_{IO_2}. In wet studies, exercise was done throughout the entire work

period. Mean oxygen consumption was about 225 ml / min higher than the work target oxygen consumption during both dry and wet experiments. Mean resting oxygen consumptions were high, 1265 ml / min, probably due to the effects of climbing into the experimental position, apprehension, physical discomfort from sitting on the bicycle ergometer or as a result of measurement errors discussed in the Methods section. Oxygen consumption did not change significantly with immersion or hyperoxia. Oxygen consumption and heart rate were significantly higher for the working condition compared to rest. Increased P_{IO_2} resulted in decreased heart rate during immersed rest and work but had no significant effect in dry experiments. Heart rate was significantly higher during work at both 0.21 and 1.75 ATA P_{IO_2}. Working heart rate during immersion was significantly lower than dry working heart rate at both P_{IO_2}'s. Mean oxygen consumption and heart rate data from Phases 9 and 10 are shown in Figure 32.

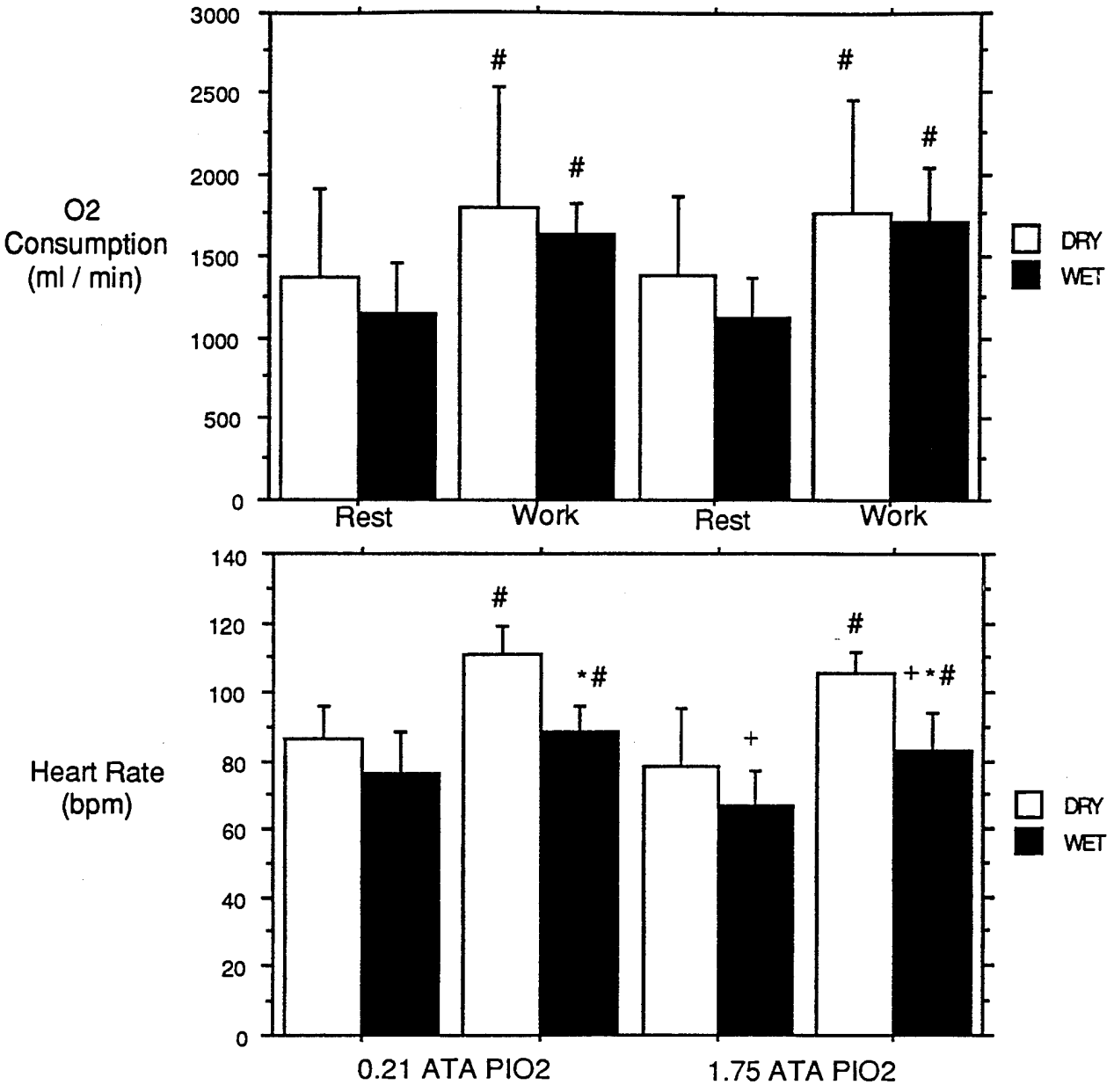

Figure 32: Mean heart rate and oxygen consumption during dry □, n = 10, and wet ■, n = 9, rest and work * Sig. diff. from dry, $p<0.05$. + Sig. diff. from 0.21 ATA PIO_2, $p < 0.05$.

Increased P_{IO_2} resulted in decreased dry resting $P_{ET_{CO_2}}$ and increased dry working $P_{ET_{CO_2}}$ but had no significant effect during immersion. Increased P_{IO_2} resulted in a significant increase in ventilation during dry rest, dry work, and immersed work. Heart rate, $P_{ET_{CO_2}}$, and ventilation were significantly higher during work at both 0.21 and 1.75 ATA P_{IO_2}. Working heart rate during immersion was significantly lower than working heart rate in the dry at both P_{IO_2}'s. Working ventilation was significantly lower during immersion than during dry

experiments at both 0.21 and 1.75 ATA P_{IO_2}. Resting ventilation was significantly higher during immersion at 0.21 ATA P_{IO_2}. $P_{ET_{CO_2}}$ measured during immersed work was significantly lower than during dry work. Mean ventilatory data from Phases 9 and 10 are shown in Figure 33.

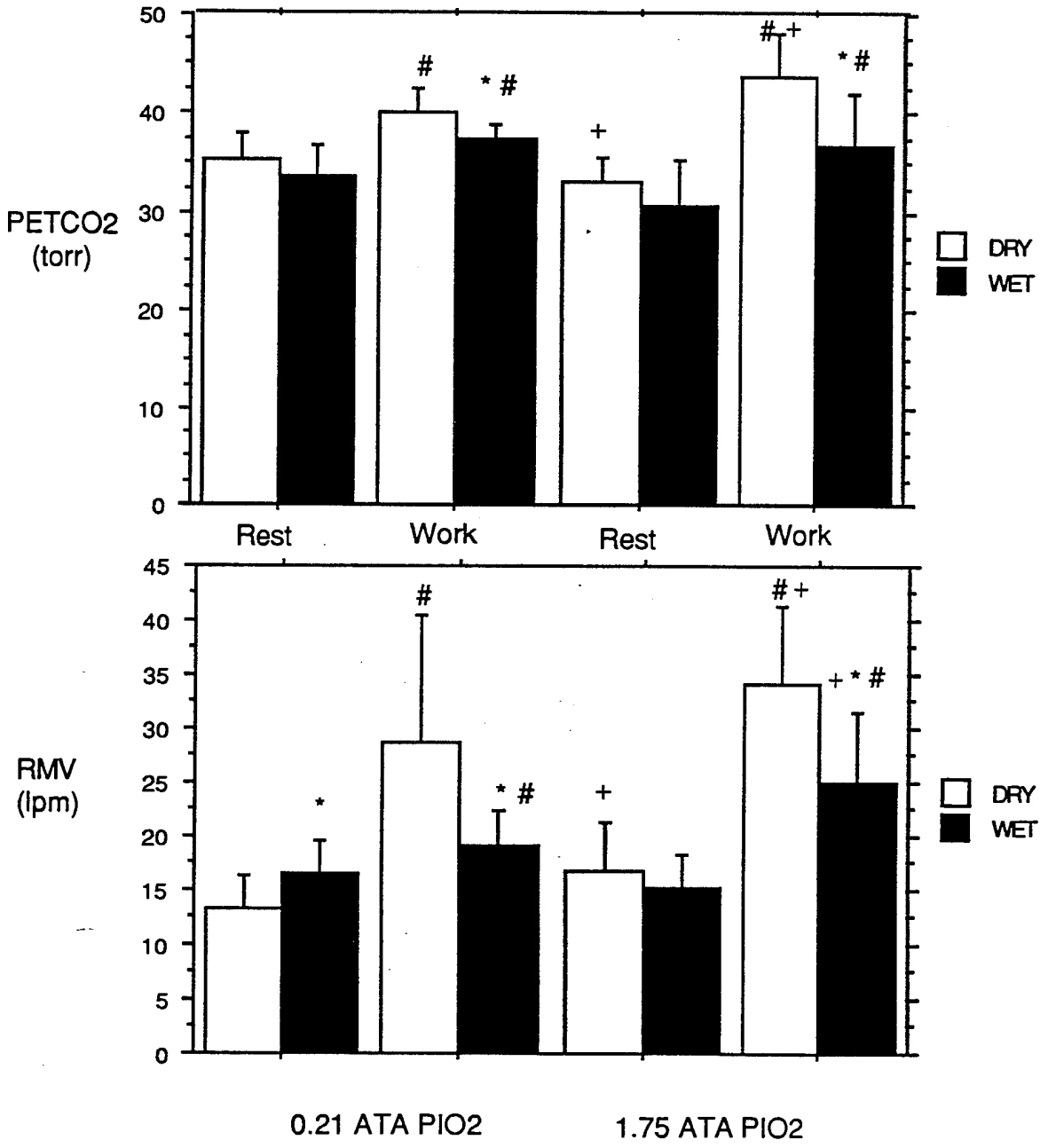

Figure 33: Mean ventilation and $P_{ET_{CO_2}}$ during dry □, n = 10, and wet ■, n = 9, rest and work: * Sig. diff. from dry, $p<0.05$. + Sig. diff. from 0.21 ATA P_{IO_2}, $p < 0.05$.

3.7.2 Cerebral Oxygenation

Mean cerebral oxygenation data are expressed as changes in NIR signals from baseline which was recorded after 5 minutes of normobaric, normocapnic, normoxia while dry. In Phases 9 and 10, changes from baseline were calculated after 10 minutes at 0.21 ATA P_{IO_2}, and 40 minutes at 1.75 ATA P_{IO_2}. Mean data from the 10 experiments in Phase 9 and the 9 experiments in Phase 10 are shown in Figure 34.

Hyperoxia decreased tHb and increased $tHbO_2$ during work in both the wet and dry environments. tBV and cyt. a,a_3 oxidation were not significantly altered by hyperoxia. Work resulting in an oxygen consumption of 1.5 lpm did not significantly change cerebral oxygenation except for increasing $tHbO_2$ at 1.75 ATA P_{IO_2} during dry experiments. Trends toward decreased tHb and increased oxidation of cyt. a,a_3 were observed with work but were not significant.

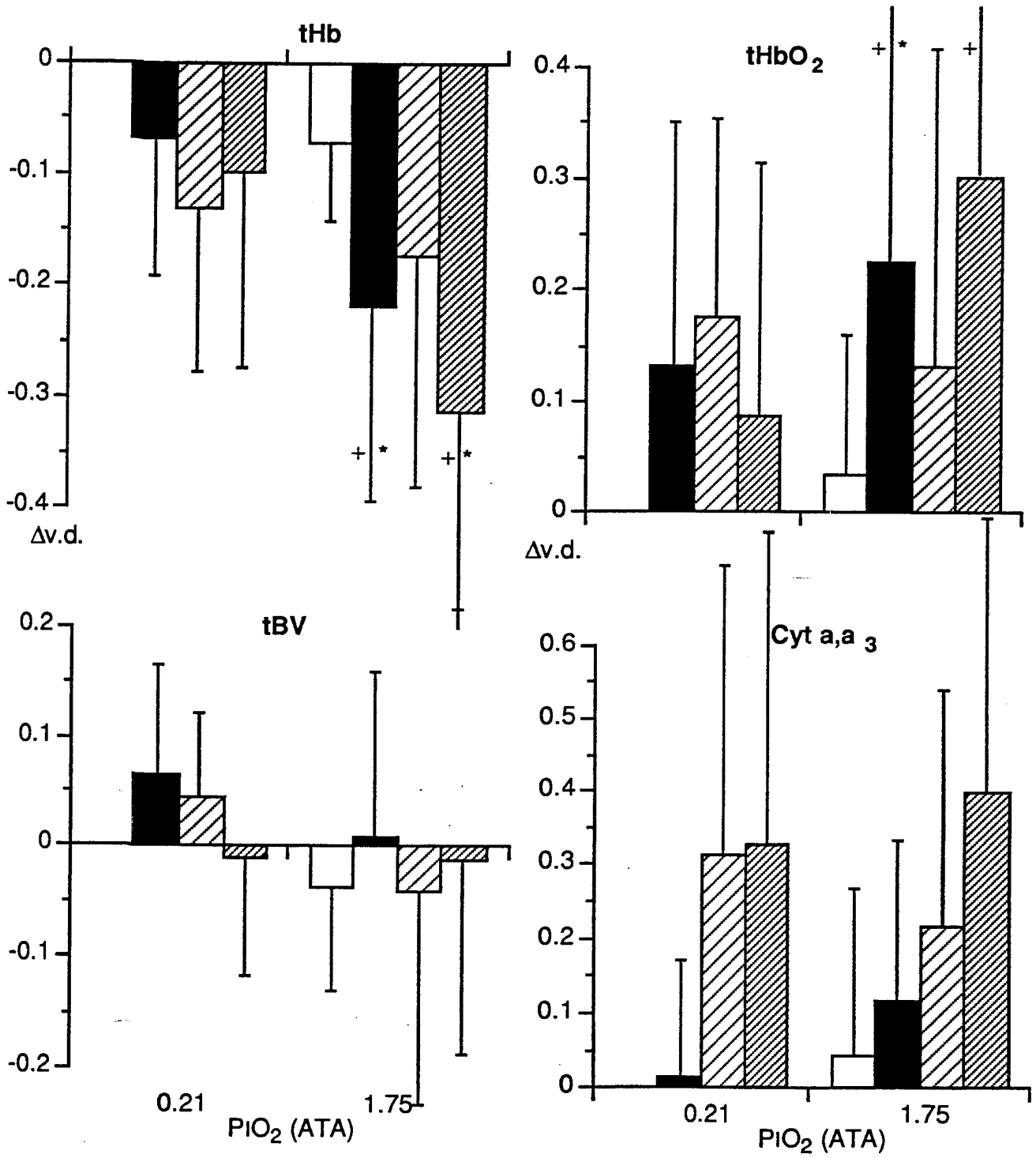

Figure 34: Mean cerebral oxygenation changes during dry and wet work. Changes in variations in density (Δvd) from dry control in brain tHb, $tHbO_2$, tBV, and oxidized Cyt a,a_3 at rest (□, ▨)and at the end of the 10 min (PIO_2 = 0.21) and 40 min (PIO_2 = 1.75) of work (■, ▨) with an o_2 consumption of 1.5 lpm. Solid bars are dry experiments and striped bares are wet experiments. * Sig. diff. from rest, $p < 0.05$. + Sig. diff. from 0.21 ATA PIO_2, $p < 0.05$. # Sig. diff. from dry, $p < 0.05$.

3.7.3 Symptoms

A symptom was reported in Phase 9, dry exercise at 1.75 ATA P_{IO_2}. The subject stopped the experiment after 20 minutes of exercise due to "air hunger" leading to nausea and anxiety. Symptoms cleared after 2 minutes of breathing air. No symptoms were reported in Phase 10, wet exercise

4. DISCUSSION

The hypotheses for this work were 1) during exposure to elevated O_2 partial pressure, inspired CO_2 and exposure duration, immersion, and work increase oxygen delivery to the brain and raise the probability that CNS oxygen toxicity will occur and 2) changes in cerebral oxygenation, ventilation, and other physiological responses can be correlated to CNS oxygen toxicity occurrence, possibly providing means for determining individual susceptibility.

We found that 40 min at 1.75 ATA P_{IO_2} and 20 min at 2.80 ATA P_{IO_2} were not sufficient to produce CNS oxygen toxicity in dry resting conditions. Performance of light work (O_2 consumption = 1.7 lpm) while dry and immersion in thermoneutral water (35° C), head-out or complete while at rest or during light work (O_2 consumption = 1.7 lpm) also failed to produce symptoms consistent with CNS oxygen toxicity during the same oxygen exposures. Acute progressive increase of inspired CO_2 and 10 min of elevated inspired CO_2 produced symptoms consistent with CNS oxygen toxicity at 2.80 ATA P_{IO_2}. Oxygen toxicity symptoms did not occur with either elevated O_2 or CO_2 alone. The results of this study suggest that inspired CO_2 is a potent instigator of CNS oxygen toxicity, more potent than oxygen alone or in combination with immersion. The role of work was not fully investigated. Measurement of ventilatory response to CO_2 with concomitant measurement of cerebral oxygenation by NIRS may lead to the determination of those most susceptible to CNS oxygen toxicity but was not highly correlated with symptom occurrence.

Oxygen becomes toxic to cells in every type of tissue given sufficient concentration and exposure duration. Oxygen in itself is not toxic but is pharmacologically active and participates in a variety of intracellular reactions which have potentially toxic effects (Clark 1982). A tissue's susceptibility to the toxic effects of oxygen is determined by its biochemical characteristics, antioxidant defenses, metabolic activities, and oxygen supply (Fife 1991a).

The CNS is among the most susceptible of all tissues (Dickens 1946; Stadie et al. 1945), but due to the presence of various protective mechanisms, regional vascular regulation, multiple concentration gradients, and high rates of oxidative metabolism the brain is protected at atmospheric pressures by limiting oxygen concentrations in cerebral tissue. It is only at hyperbaric pressures that oxygen concentrations in the brain may approach concentrations which cause toxicity at ambient pressure in other tissues, e.g. the lungs. Toxic oxygen concentrations in cerebral tissue are produced by increased oxygen delivery to the brain.

4.1 Symptoms

In the 133 hyperoxic exposures ($P_{IO_2} \geq 1.75$ ATA) in Phases 1 through 10, symptoms of CNS oxygen toxicity were reported in 35 including one oxygen seizure. Symptoms can be divided into two groups, based on classifications proposed by Butler and Thalmann (1984), definite (tinnitus, tunnel vision, disorientation, muscle twitching, and incoordination) and probable (light-headedness, apprehension, dysphoria, and lethargy) . Probable symptoms were classified as definite if they were severe. Thirteen of 35 symptom reports were classified as definite while the 20 were called probable or minor and 2 were termed possible, a classification used if other explanations were likely but not incontrovertable.

In Phase 1, definite symptoms (tinnitus, tunnel vision, and extreme anxiety) were reported in 7 of 34 experiments. These symptoms match the Butler and Thalmann classification if extreme anxiety is termed severe apprehension. Other symptoms (narcosis, light-headedness, dizziness, numbness, tingling, headache, and sweats) were reported in 14 other experiments. If narcosis is termed dysphoria and dizziness is called light-headedness, this set of symptoms is similar to the probable symptom classification of Bulter and Thalmann with the exception of numbness, tingling, headache, and sweats. These are not typical symptoms of CNS oxygen toxicity and may have arisen from the combination of oxygen and carbon dioxide stressors. The ambiguity of these symptoms caused us to use the term minor instead of probable in the Phase 1 report (Appendix A). Of the 21 experiments with reported

symptoms, 15 experiments had reports of multiple symptom occurrence.

In Phase 2, definite symptoms (tinnitus, tunnel vision, and extreme anxiety), were reported in 6 of 26 experiments. Minor symptoms (nausea, narcosis, and light headedness) were reported in 4 other experiments. The only difference between Phase 1 and the dry portion of Phase 2 was body position, semi-recumbent vs seated upright. The greater incidence of minor symptoms in Phase 1 (14 of 34) compared to Phase 2 (2 of 13) could have been the result of hemodynamic differences due to experimental body position or the result of the greater sensitivity to minor physiological disturbances afforded by the more relaxed experimental position in Phase 1. In Phase 3, there was a convulsion and a definite symptom (extreme anxiety).

Another classification, possible, is used in this report to describe symptom reports which have possible explanations other than oxygen toxicity. One possible symptom (extreme anxiety) was reported in Phase 7 where head-out and total immersion were compared in the same experiment, but the source of the anxiety could have been the high negative static lung load mentioned by the subject. One possible symptom was reported in Phase 9, nausea, but was unusual because it started with the sensation of heart burn and air hunger and lead to nausea possibly due to indigestion which did not clear entirely after the experiment. Both possible symptoms resulted in subjects dropping out from the remainder of the scheduled experimentation.

It is well established that given sufficient oxygen pressure and exposure duration, CNS related symptoms and tonic/clonic oxygen seizures, indistinguishable from grand mal seizures, result in man (Behnke 1935, Donald 1942). The exact mechanism(s) which initiate seizures have not yet been determined. It is generally accepted that the initial event in the development of oxygen toxicity is increased production of oxygen free radicals which follows from increased oxygen delivery (Gerschman, 1964, Yusa, et al, 1987). The formation of free radicals has been shown to cause complex biochemical changes in neuronal tissue including inactivation of intracellular enzymes (Haugaard, 1968), increased formation of lipid

hydroperoxides (Noda, et al, 1983; Jerrett, et al, 1973), changes in the balance of cerebral neurotransmitters such as GABA depletion (Wood, et al, 1971), Na^+-K^+ ATPase oxidation (Gottlieb, et al, 1976), decreased mitochondrial respiration rate leading to decreased ATP availability (Woodhall, et al, 1971) and inactivation of membrane transport systems (Kovachich and Haugaard, 1981). Biochemical changes occur upon increase in cellular oxygen tension resulting in alterations in cellular metabolism. If oxygen tension has been increased in a large enough area and for sufficient duration to overcome antioxidant defenses, enough cellular changes occur in the brain to inhibit normal function leading to symptoms or convulsions (Haugaard, 1968; Lambertsen, 1978).

The delay from the initial hyperoxic exposure to the appearance of symptoms depend on individual susceptibility and oxygen delivery. Physiologic factors such as prevailing adrenergic tone and endocrinologically mediated stress reactions may alter oxygen seizure thresholds. Factors which increase metabolic rate above normal such as fever, hyperthermia, hyperthyroidism or an increase in catecholamines can increase the risk of oxygen toxicity, while hypothyroidism, starvation, and hypothermia decrease risk (Fife 1991a).

4.1.1 P_{IO_2}

In the present study, 1.75 and 2.80 ATA P_{IO_2} were tested because a P_{IO_2} of 1.75 is equivalent to 100% oxygen at 25 fsw which is the depth of the longest exposure (240 min) allowed by the US Navy and 2.80 ATA P_{IO_2} is equivalent to 100% oxygen at 60 fsw which is the exposure pressure for Treatment Tables 5 and 6. An inverse hyperbolic relationship exists between inspired oxygen pressure and exposure duration to produce symptoms of CNS oxygen toxicity (Dickens, 1962; Clark, 1974).

In this study, symptoms were reported at 2.80 ATA P_{IO_2} (103 exposures) in 34 of the 35 experiments with symptom reports, though 33 reports occurred while CO_2 was inspired. Others have reported symptoms (8 of 113 exposures) in resting immersed dives at 2.80 ATA P_{IO_2} in 18° and 32° C water at exposure durations of 12 - 76 min (Donald, 1947; Yarborough,

1947). A single possible symptom was reported in experiments testing exposure duration at rest for 30 min at 2.80 ATA P_{IO_2} (10 min head-out immersed, 10 min fully immersed, and 10 min head-out recovery). The possible symptom was reported 5 min into the total immersion portion of the experiment at 2.80 ATA P_{IO_2}. No symptoms were reported in 23 other 20 min exposure duration experiments at 2.80 ATA P_{IO_2} (11 dry, 6 head-out immersed, and 6 fully immersed).

The one experiment resulting in a possible symptom report (air hunger / nausea) at 1.75 ATA P_{IO_2} (133 exposures) occurred after 20 min of dry work (oxygen consumption = 1.5 lpm). No symptoms were reported by Clark et al. (1995) for 10 subjects doing dry 120 min work/rest dives at 2.0 ATA P_{IO_2}. Others have reported symptoms at 1.75 ATA P_{IO_2} during working immersed dives in 18°, 21°, and 22° water at 72 - 178 min in 7 of 90 exposures (Donald, 1947; Piantadosi, 1980; Butler and Thalmann, 1986).

4.1.2 CO_2

Inspired CO_2 during oxygen breathing increases cerebral blood flow (Kety and Schmidt, 1948) and increases oxygen delivery to the brain (Lambertsen et al, 1956). Failure of oxygen induced vasoconstriction, as a result of tissue CO_2 tension increase or ischemic injury (Lambertsen et al., 1956; Miller et al, 1970), has been shown to occur before the onset of seizures (Bean, Lingnell, and Burgess, 1972). In Lambertsen's study, subjects breathed 7% surface equivalent (SEV) CO_2 in 3.5 ATA oxygen (5 exposures) with all experiments ending with symptom appearance (mean time 7.8 min). In the current study, most symptom reports, 33 of the 35 at 2.80 ATA P_{IO_2} (70 exposures), occurred during exposures to increased inspired CO_2. 31 of the 33 were associated with $P_{ETCO_2} > 57$ torr (60 exposures). Inspired CO_2 gradually rose to 7 % sev at 2.80 ATA P_{IO_2} over 7 min. One symptom report occurred in the last minute of a 10 minute exposure to $P_{ETCO_2} = 50$ torr (inspired CO_2 was 5.5% sev) at 2.80 ATA P_{IO_2} (10 exposures). The only convulsion occurred 1 minute after a 10 minute exposure to $P_{ETCO_2} = 50$ torr. No symptoms were reported during inspired CO_2 exposures at 1.75 ATA P_{IO_2}.

4.1.3 Immersion and Static Lung Load

No immersion effect was found in the current study in relation to symptom incidence. In Phase 2 of the current study where inspired CO_2 was allowed to increase to 7.5 % surface equivalent, 5 symptom reports were made during head-out immersion. This was the same symptom incidence as in dry experiments under otherwise similar conditions. No symptoms were reported during head-out immersion without inspired CO_2. Only one symptom was reported during total immersion without inspired CO_2. The subject was head-out immersed at rest breathing gas with a PIO_2 of 2.80 ATA for 10 min then totally immersed , and became very anxious after 5 min and stopped the experiment. The subject complained of a large negative static lung load (~ -45 cm H_2O). Negative static lung load has been shown to increase dyspnea (Lundgren, 1984) but has little measurable effect on metabolic and ventilatory parameters during submerged exercise (Thalmann et al, 1979).

The delay to onset of symptoms of CNS oxygen toxicity has been shown to decrease with total immersion (18 °C) (Donald, 1947). Butler and Thalmann (1986) found a greater incidence of symptoms in immersed (22 °C) exercising divers than Clark et al (1995) found in dry exercising divers under similar conditions.

4.1.4 Work

In the present study, a single symptom report was made during dry work, after 20 min with an oxygen consumption of 1.7 lpm, at 1.75 ATA PIO_2. Time constraints prevented working studies to be performed at 2.80 ATA PIO_2. The delay to onset of symptoms of CNS oxygen toxicity has been shown to decrease with exercise (Donald, 1947; Yarbrough et al, 1947; Butler and Thalmann, 1984, 1986). Even in the absence of hypercarbia due to exercise, symptoms are more likely to appear in an exercising diver than a diver at rest (Donald, 1947).

No symptoms of CNS oxygen toxicity were reported during dry exercise at 2.0 ATA PIO_2 (Lambertsen, 1959; Clark et al, 1995) while symptoms were reported during immersed working dives at 2.06 ATA PIO_2 (Butler and Thalmann, 1986). Symptoms have been

reported at 1.75 ATA P_{IO_2} during working immersed dives in 18°, 21°, and 22° water at 72 - 178 min in 7 of 90 exposures (Donald, 1947; Piantadosi, 1980; Butler and Thalmann, 1986).

4.2 Cerebral Oxygenation

Near-infrared spectroscopy (NIRS) allows for the assessment of tissue oxygenation by measuring relative changes in the amounts of oxyhemoglobin ($tHbO_2$) and deoxyhemoglobin (tHb) (and total blood volume, tBV, by the arithmetic sum assuming constant hematocrit) and the redox state of cytochrome a,a_3 (cyt. a,a_3) in the illuminated area. In this study, NIRS was applied to illuminate the frontal parietal cerebral cortex. Changes from baseline in the amount of tHb, $tHbO_2$, tBV, and cyt. a,a_3 were measured for a half annulus of tissue extending 3.5 cm from light entry to exit to a depth of approximately 2 cm though neither the exact dimensions of the illuminated area nor the exact pathlength that the photons travel are known and thus the concentrations of absorbing chromophores cannot be determined. A separation of 3.0 cm from transmitting to receiving optrode is sufficient to ensure superficial tissue layers contribute least to absorption changes (Patterson et al, 1989). Gray matter contributes more to optical absorption changes than white matter in the illuminated tissue volume (Eggert and Blazek, 1987). The contribution of the skin and bone overlying the illuminated area has been estimated to be 5% of the total signal in small animals (Piantadosi et al, 1986) and for skin in humans (Hampson and Piantadosi, 1988). The contribution of blood within the skull of humans has not been determined. The blood volume of the tissue in the illuminated area has been estimated as 5% of the total tissue volume where capillaries are the predominant vessels (Piantadosi, 1993) and venous vessels are more numerous than arterial vessels (McCormick et al, 1992).

Increases in the tHb signal result from the following conditions : 1) a decrease in blood oxygen saturation, 2) a decrease in cerebral venous return or 3) increased oxygen consumption.

Increases in the $tHbO_2$ signal result from the following conditions : 1) an increase in

blood oxygen saturation, 2) increased blood flow or 3) decreased oxygen consumption.

Increases in the tBV signal result from the following conditions : 1) a decrease in cerebral venous return or 2) increased blood flow. Changes in tBV are linearly related to changes in regional blood flow (Colacino et al, 1981; Pryds et al, 1990).

Increases in the oxidized cyt. a,a_3 signal result from the following conditions : 1) increased tissue PO_2 , 2) decreased cellular substrate concentration leading to a lack of electrons required for oxidative cellular respiration 3) increased oxygen consumption with an adequate oxygen supply (Brazy, 1991).

tHb, $tHbO_2$, and tBV signals relate regional cerebral blood supply information while the cyt. a,a_3 redox signal relates cerebral tissue metabolic information. Cyt. a,a_3 is the terminal member of the electron transport chain which occurs in the mitochondria within cells. Mitochondrial density within neurons of gray matter is very high (Wong-Riley, 1989). This enzyme directly accounts for 90% of brain oxygen utilization resulting in ATP formation necessary for cellular function. The redox state of cyt. a,a_3 in the illuminated area changes as the ratio of cyt. a,a_3 receiving electrons (reduced state reflecting substrate sufficiency) to cyt. a,a_3 giving up electrons to oxygen (oxidized reflecting oxygen sufficiency) changes. This ratio is normally constant while the flux of electrons and oxygen is relatively high. Because the substrate level is quite adequate in healthy brain tissue, the redox state of cyt. a,a_3 is an excellent marker for cellular oxygen supply and since oxidative metabolism and neuronal function are tightly coupled cyt. a,a_3 is an endogenous marker for neuronal activity (Wong-Riley, 1989; Wyatt et al, 1986). NIRS has been utilized to examine cerebral oxygenation changes during mental tasks (Hoshi et al, 1994).

4.2.1 $P_{I_{O_2}}$

In Phase 1, tHb and tBV increased and $tHbO_2$ and cyt. a,a_3 oxidation decreased with decreased $P_{I_{O_2}}$. These findings are consistent with the results of earlier studies done at this laboratory with the same NIRS instrument (Hampson et al, 1990). tHb and tBV decreased and $tHbO_2$ and cyt. a,a_3 oxidation increased with increasing $P_{I_{O_2}}$ without inspired CO_2. In

Phase 2, the cerebral oxygenation results were the same as in Phase 1 with the exception of the tBV data which showed the same trend toward vasoconstriction with hyperoxia but not significantly. The difference between the two phases was body position, recumbent in Phase 1 and seated upright in Phase 2. The normocapnic oxygen breathing portion of Phase 3 showed the same cerebral oxygenation results as found in Phase 2. The same results were found in Phase 4 where the effect of hyperoxia and exposure duration were tested during dry rest. tHb and tBV decreased though (not significantly) and $tHbO_2$ and cyt. a,a_3 oxidation increased at both 1.75 and 2.80 ATA PI_{O_2} without inspired CO_2. Significant changes in cerebral oxygenation did not occur between 1.75 and 2.80 ATA. As exposure duration increased the cerebral oxygenation changes tended to intensify but significant results were only obtained for tHb and $tHbO_2$ after 40 min at 1.75 ATA PI_{O_2}. PET_{CO_2} was significantly decreased at the end of both oxygen exposures 1.75 and 2.80 ATA PI_{O_2}. PET_{CO_2} is a good indicator of P_aCO_2 though not an equivalent measure (Young et al, 1991).

The cerebral oxygenation data from dry resting studies suggest that breathing 1.75 ATA of oxygen causes cerebral vasoconstriction with concomitant increases in cerebral blood oxygenation via increased saturation of venous blood and decreased blood CO_2 and increased cerebral tissue oxygenation as evidenced by increased cytochrome a,a_3 oxidation or availability of mitochondrial oxygen. Increasing exposure duration tends to increase both blood and tissue oxygenation as does increasing the PI_{O_2} to 2.80 ATA. The fact that tBV drops with hyperoxia did not reach significance in some Phases of the study while reaching significance in others may have been the result of differences in body position, Phase 1 to Phases 2-10, or individual variation in cerebral responses to hyperoxia. Donald (1947) made the observation that there was great variability between subjects and for the same subject over time in regards to susceptibility to CNS oxygen toxicity symptoms. We have found a large variability between subjects and by the same subject in regards to measurements of cerebral oxygenation.

Lambertsen (1953) found decreased cerebral blood flow, increased arterial and venous

saturation, and decreased P_aCO_2 during oxygen inhalation at both 1 ATA and 3.5 ATA with greater changes at 3.5 than at 1 ATA in dry resting supine subjects. Cerebral oxygen consumption did not change with oxygen inhalation. Lambertsen hypothesized that hyperoxia induced arterial hypocapnia which leads to cerebral vasoconstriction. Hyperoxia increases the amount of oxygen dissolved in plasma. More hemoglobin remains oxygenated than during normoxia because dissolved oxygen is preferentially utilized by the tissues. During air breathing, O_2 is released from hemoglobin and deoxygenated hemoglobin (Hb) is reduced during the dissociation of carbonic acid into hydrogen ions and bicarbonate, the form in which most CO_2 is transported. CO_2 is also transported via carbamino compounds, which are more readily formed by Hb than HbO_2. During hyperoxia, hemoglobin reduction is decreased and the amount of HbO_2 increased (Salzano, 1980). Therefore, hemoglobin does not fulfill its normal role in CO_2 transport, increasing Pt_{CO_2} up to 5 torr above normal and decreasing Pa_{CO_2} which causes vasoconstriction (Lambertsen, 1978).

Vasoregulation is affected by both O_2 and CO_2 tension. Cerebral blood flow is inversely related to blood oxygen content. The mechanism by which oxygen exerts its regulatory effect is unknown (Jackson, 1987). Cerebral vasodilatation in response to hypoxia is generally accepted to be mediated locally, though the exact mechanism for local cerebrovascular regulation has not been completely elucidated (Traystman and Fitzgerald, 1981). Vasoconstriction in response to hyperoxia is also thought to have a local control component since oxygen decreases vessel lumen size *in vitro* (Plewes and Farhi, 1983). Although, hyperoxia causes cerebral vasoconstriction (Bean et al, 1972), cerebral tissue PO_2 increases (Jamieson and van den Brenk, 1963; Torbati et al, 1977). These findings are corroborated by the present study since cerebral cytochrome oxidation increased with exposure to 1.75 and 2.80 ATA PI_{O_2}. The redox state of cytochrome a,a_3 is an indicator of cerebral oxidative metabolism and is a nearly continuous function of oxygen concentration from hypoxia to normoxia (Hampson et al, 1990). The basal redox state of cyt. a,a_3 is not known although it has been shown to be less than fully oxidized (Jobsis et al, 1988) and studies in anesthetized

animals suggest cytochrome a,a_3 is 54% oxidized in normoxia (Sylvia et al. 1984) while *in vitro* studies have shown nearly complete oxidation of cytochrome a,a_3 (Chance and Williams, 1956). Increases in cytochrome oxidation with hyperoxia may indicate increased cerebral metabolism although studies have shown cerebral ATP levels remain constant during hyperbaric oxygenation (Nolan and Faiman, 1974). Oxidation of NADH and increased electron flow rate through the respiratory chain has been found during hyperbaric oxygen exposure (Chance et al, 1965). and cyt. a,a_3 has been shown to become continuously more reduced with increasing hypoxia in awake humans (Hampson et al, 1990) and more oxidized during CO_2 inhalation with hyperbaric oxygen (Hempel et al, 1977).

Increased partial pressure of oxygen decreased heart rate during head-out and total immersion for resting and working divers. Bradycardia has been shown with increasing $P_{I_{O_2}}$ in resting and working divers (Fagraeus, 1974; Whalen et al, 1965). Increased gas density and pressure may contribute to hyperbaric bradycardia (Flynn et al, 1972).

4.2.2 $\underline{CO_2}$

In Phases 1 and 2 of our study, rebreathing CO_2 to $P_{ET_{CO_2}}$ = 60 torr (~ 7.5% SEV inspired CO_2) increased $tHbO_2$ significantly and decreased tHb significantly. In Phase 1, we found that cytochrome a,a_3 oxidation did not change significantly at maximum CO_2 despite a significant vasodilatation at each $P_{I_{O_2}}$. In Phase 2, a repeat of the initial study, except for body position, we found that cyt. a,a3 oxidation did increase significantly at maximum CO_2 despite an insignificant rise in tBV at 2.80 ATA. Increases in cyt. a,a_3 oxidation were accompanied by significant vasodilatation at 0.21 and 1.75 ATA $P_{I_{O_2}}$. A significant decrease in tHb and significant increases in $tHbO_2$ and cyt. a,a_3 oxidation were recorded in Phase 3 when rebreathing to $P_{ET_{CO_2}}$ = 50 torr at each $P_{I_{O_2}}$. Significant vasodilatation occurred upon CO_2 rebreathing at 0.21 and 2.80 ATA $P_{I_{O_2}}$. These results suggest that hyperoxic cerebral vasoconstriction is released by CO_2 inhalation at $P_{I_{O_2}}$'s $\leq$ 1.75 ATA in resting subjects seated upright but vasoconstriction at 2.80 ATA was not reversed significantly.

Differences between Phases 1 and 2 may be attributed to the difference in body position

during these experiments. Phase 1 subjects were recumbent while Phase 2 subjects were seated upright. Cerebral blood flow is lower in the seated upright position than in the recumbent position due to a decrease in the arteriovenous pressure gradient while cerebral vascular resistance remains constant (Patterson and Warren, 1952). Starting at a higher flow condition during recumbence may have allowed for the significant drops in tBV during hyperoxia without inspired CO_2 and the significant increases during CO_2 rebreathing. Lambertsen (1956) found increased cerebral blood flow in supine resting subjects at 3.5 ATA PI_{O_2} with 7% inspired CO_2 and all subjects had symptoms of CNS oxygen toxicity. In Phase 1 experiments of the current study, in which subjects were resting, dry, and recumbent, a greater incidence of symptoms occurred 21/34 (62%) compared to Phase 2 where subjects were resting, dry, and seated upright, 5/13 (38%).

Hempel et al (1977) found marked vasodilatation and maximal cyt. a,a_3 oxidation in anesthetized cats breathing 5% CO_2 - 95% O_2 at 4 ATA using NIRS. CO_2 is a powerful cerebral vasodilator (Kety and Schmidt, 1947). CO_2 produces vascular changes by altering perivascular and intracellular smooth muscle pH, although the mechanism of vasoregulation by pH and CO_2 is unclear (Berne 1986). Lambertsen (1955) showed that cerebral venous oxygen content remained relatively constant for PI_{O_2}'s of 0.21 or 3.5 ATA, but when PI_{CO_2} was raised to 53 torr, the venous oxygen content increased by 1000%, decreasing arteriovenous oxygen difference, and drastically elevating cerebral oxygenation (Lambertsen, 1956). Cerebral oxygen metabolism is maintained at a constant level for P_tO_2 from 50 torr to those which precipitate oxygen toxicity (Lambertsen, 1978). Cerebral metabolic oxygen rate is maintained via balancing of cerebral blood flow, which is directly related to blood volume, and cerebral arteriovenous (AV) oxygen difference (Piantadosi, 1989b).

In Phase 3 of the current study, exposure duration for inspired CO_2 breathing was tested. tHb was significantly lower and $tHbO_2$ and cyt. a,a_3 oxidation were significantly higher at each PI_{O_2} after 10 min of rebreathing at PET_{CO_2} = 50 torr (~ 5.5% inspired CO_2). tBV rose significantly with increased exposure duration at 0.21 ATA PI_{O_2} only.

4.2.3 Immersion and Static Lung Load

Initially in Phases 5 and 6, head-out and total immersion were tested in separate experiments to determine the effect of immersion in thermoneutral water on cerebral oxygenation and symptoms incidence. Head-out and total immersion showed similar cerebral oxygenation changes to dry exposures with the following exceptions. Hyperoxic vasoconstriction reached significance after 20 min of head-out immersion at 2.80 ATA PI_{O_2}. Cytochrome oxidation increase reached significance after 20 min of total immersion at 2.80 ATA PI_{O_2}. In Phase 7, the 2 types of immersion were compared directly at 0.21 and 2.80 ATA PI_{O_2}. Essentially facial immersion was tested and found to cause decreased tHb and increased $tHbO_2$, tBV, and cyt. a,a_3 oxidation. Due to the initial experimental setup, a negative static lung load was present during total immersion. The test was repeated at 0.21 ATA PI_{O_2} with a positive static lung load in Phase 8, with the same results. The negative static lung load had a significantly greater effect on cerebral oxygenation than the positive static lung load.

Head-out immersion in thermoneutral water (35° C) results in 1) increased cardiac stroke volume with unchanged heart rate leading to increased cardiac output 2) a cephalic redistribution of circulating blood volume and 3) diuresis compared to dry conditions (Yin, 1984). Head-out immersion is more similar to the dry recumbent position than to dry seated-upright. This is borne out in the similarities in the tBV signal changes with increasing PI_{O_2} in Phases 1 and 5. Head-out immersion creates a negative static lung load of -30 cm H_2O (Lundgren, 1984). The effect of head-out immersion on cerebral circulation has not been reported, though peripheral perfusion to muscles increases (Arborelius et al, 1972). Total immersion created a negative static lung load of -45 cm H_2O.

The effect of total immersion on cerebral circulation has not been reported but is thought to increase cerebral blood flow as part of the human dive reflex. Responses to facial immersion and apnea, the dive reflex, include bradycardia and peripheral vasoconstriction. These responses are thought to be part of an adaptive oxygen conservation reflex which

redistributes blood flow to the brain and heart (Irving, 1938). In Phase 8, facial immersion increased cerebral oxygenation by increasing $tHbO_2$, tBV, and cytochrome a,a_3 oxidation. Apnea further increased cerebral oxygenation during facial immersion but not during head-out immersion. Significant bradycardia occurred during breath holding but not during facial immersion. Total immersion has been shown to increase heart rate (Lanphier and Camporesi, 1982). $P_{ET}CO_2$ was unchanged with facial immersion. In Phase 7, increased cerebral oxygenation with facial immersion was measured at both 0.21 ATA and 2.80 ATA PI_{O_2}. Heart rate decreased significantly with hyperoxia as has been previously been shown in both animal and human studies (Bean and Siegfried, 1945; Lambertsen, 1953; Wood et al, 1967). In Phase 8, increased cerebral oxygenation was measured during breathing with a positive static lung load though the increase was significantly lower than during the negative static lung load breathing of Phase 7.

4.2.5 Work

Work decreased tHb and increased $tHbO_2$ significantly at 1.75 ATA PI_{O_2} and tended to increase cyt. a,a_3 oxidation but not significantly for both dry and totally immersed conditions. $P_{ET}CO_2$ was significantly increased by work at both 0.21 and 1.75 ATA PI_{O_2}. $P_{ET}CO_2$ was significantly lower during wet work compared to dry work as was ventilation and heart rate possibly due to slightly lower oxygen consumption during immersion. Exercise during immersion was subjectively easier and more comfortable than dry work, probably a result of buoyancy effects.

Work increases arterial PCO_2 during hyperoxia in both dry and immersed conditions (Stevens et al, 1990; Salzano et al, 1967). Increased arterial PCO_2 leads to cerebral vasodilatation as discussed above. Lambertsen (1959) found that dry exercise while breathing air or hyperbaric oxygen at 2.0 ATA PI_{O_2}, oxygen consumption = 2 lpm, caused no change in cerebral circulation. Immersed exercise may lead to CO_2 retention in divers whose alveolar ventilation is abnormally low leading to abnormally high arterial PCO_2 for the level of exercise undertaken (Miller et al, 1971; Lanphier and Camporesi, 1982).

4.3 Ventilation

Arterial partial pressures of O_2 and CO_2 are the major signals in ventilatory control. There are mechanical, neural, and chemical influences but P_aCO_2 and P_aO_2 are the major contributors. Arterial oxygen and carbon dioxide are sensed in the carotid bodies but P_aCO_2 has a larger effect on receptors in the medulla oblongata. Carbon dioxide response is assessed by having subjects inhale CO_2 and a constant PIO_2 while measuring ventilation changes due to increasing P_aCO_2. Oxygen response is usually assessed by maintaining a constant P_aCO_2 and measuring ventilation changes to decreases in arterial oxygen. Ventilatory response to CO_2 is greater in magnitude than ventilatory response to oxygen. Ventilatory response to CO_2 is essentially linear and sensitive compared to ventilatory response to oxygen which is hyperbolic and insensitive, except at very hypoxic levels, to perturbations (Anthonisen, 1984).

We measured ventilatory responses to CO_2 in Phases 1 - 3 of the present study. Response to oxygen was not measured. Our rebreathing method used to determine ventilatory response differed from clinical methods, in which rebreathing begins with 7% CO_2, nearly matching normal Pv_{CO_2} at 50 torr (Read, 1967). In clinical studies, ventilatory response to CO_2 with a PI_{O_2} of 0.21 ATA ranged from 0.56 to 8.16 lpm $Torr^{-1}$, in healthy subjects, although 80% had responses between 1.00 and 4.00 lpm·$torr^{-1}$ (Rebuck and Read, 1971). Sex, genetic factors, personality, lung size, and mental activity affect ventilatory response. The coefficient of variation of ventilatory response for a subject tested repeatedly, 2 - 6 times, on the same day ranged from 2 to 50% (Rebuck and Slusky, 1981).

We obtained similar results in our studies at the surface despite methodological differences. In Phase 1, ventilatory responses ranged from 0.66 to 6.07 lpm·$torr^{-1}$ with 88% of responses between 1.00 and 4.00 lpm·$torr^{-1}$. In repeat experiments, 2 to 8 times, weeks or months apart, we noted coefficients of variation ranging from 2 to 46%. In Phase 2, ventilatory responses ranged from 0.34 to 4.94 lpm·$torr^{-1}$ with 62% of responses between 1.00 and 4.00

lpm·torr^{-1}. In two sets of repeat experiments, weeks apart, we noted coefficients of variation ranging from 3 to 37%. In Phase 3, ventilatory responses ranged from 0.60 to 3.27 lpm·torr^{-1} with 80% of responses between 1.00 and 4.00 lpm·torr^{-1}. In Phase 1 and 2, ventilatory responses were calculated for rebreathing to 60 torr $P_{ET}CO_2$ while Phase 3 ventilatory responses were calculated for rebreathing to 50 torr $P_{ET}CO_2$.

4.3.1 $\underline{PIO_2}$

We observed reduced ventilatory response to CO_2 with increasing PIO_2 (Phases 1 and 2). Hyperoxia depresses central respiratory control leading to reduced response to CO_2 (Lambertsen, 1978). We observed either no significant change or an increase in ventilation with increasing PIO_2 during dry rest (Phases 3, 4, and 9) but P_aCO_2 was not controlled. At a given oxygen consumption, P_aCO_2 is inversely proportional to ventilation (Anthonisen, 1984). Lambertsen (1956) showed that oxygen consumption does not change with increased PIO_2. We observed a significant decrease in $P_{ET}CO_2$ with increasing PIO_2 during dry rest (Phases 3, 4, and 9) which may be explained by hyperventilation which was significant in some cases (Phase 9). Acute exposure to hyperoxia, < 3 ATA, has been shown to reduce afferent carotid chemoreceptor activity reducing ventilation but at the same time hyperoxia raises brain P_tCO_2 increasing ventilation (Salzano et al, 1984). The latter effect seems to dominate via the following mechanism: hyperoxia increases dissolved oxygen content while hemoglobin remains saturated, reducing its effectiveness as an aid in the transport of CO_2 to the lungs. This raises P_tCO_2 up to 5 torr over normoxia, increasing ventilation slightly (Lambertsen, 1978). Hyperoxic exposure durations of 20 min at 2.80 PIO_2 ATA and 40 min at 1.75 ATA PIO_2 did not significantly alter ventilation.

4.3.2 $\underline{CO_2}$

Rebreathing was used to progressively increase inspired CO_2. Ventilation increased linearly during rebreathing and ventilatory response to CO_2 was measured as discussed in the previous section. We observed a significant decrease in $P_{ET}CO_2$ with increasing PIO_2 during dry rest (Phases 3, 4, and 9). 10 min of hypercapnic exposure duration of $P_{ET}CO_2$.= 50

torr did not significantly alter ventilation.

4.3.3 Immersion

Head-out immersion had no significant effect on ventilatory response to CO_2 (Phase 2). Immersion creates an increased work of breathing due to the combined effects of hydrostatic pressure imbalance, increased pulmonary resistance, and reduced compliance. (Morrison and Taylor, 1990). Head-out immersion decreases total lung capacity, vital capacity, functional reserve capacity (Farhi and Linnarsson, 1977), and lung compliance (Dahlback et al, 1978,1979) and increases closing volume (Bondi, 1976), and flow resistance (Flynn et al, 1975). Most investigators show no change or small changes in ventilatory parameters with head-out immersion (Lanphier and Camporesi, 1982). We found that total immersion reduced $P_{ET_{CO_2}}$ and ventilation during work compared to dry conditions. Thalmann et al. (1979) found that total immersion reduced ventilation at a given oxygen consumption.

4.3.4 Work

The hyperventilation hypocapnia during dry resting breathing of 2.0 ATA $P_{I_{O_2}}$ described by Gelfand et al (1987) was observed in the current study at 1.75 ATA $P_{I_{O_2}}$ while dry but not during total immersion. Light work resulting in a 500 ml / min increase in estimated oxygen consumption over rest, not only increased ventilation at both 0.21 and 1.75 ATA $P_{I_{O_2}}$ in dry and totally immersed conditions but also resulted in increased $P_{ET_{CO_2}}$. Clark et al (1995) and Salzano et al (1967) found increased P_{CO_2} in dry working subjects breathing 2.0 ATA $P_{I_{O_2}}$ and increased alveolar and arterial P_{CO_2} have been measured at 0.21 ATA $P_{I_{O_2}}$ during light work (Dejours, 1964). Others have found elevated $P_{ET_{CO_2}}$ during exercise while breathing O_2 at 1.0 ATA (Kerem et al, 1980). Ventilation and $P_{ET_{CO_2}}$ during immersed work were lower than during dry work at 0.21 and 1.75 ATA $P_{I_{O_2}}$. Thalmann et al (1979) found lower ventilation during immersed work compared to dry but no change in $P_{ET_{CO_2}}$. Heart rate decreased with increased $P_{I_{O_2}}$ both at rest and with exercise, significantly with immersion. Clark et al (1995) found decreased heart rate during dry exercise at 2.0 ATA $P_{I_{O_2}}$ compared to 0.21 ATA $P_{I_{O_2}}$. Thalmann et al (1979) found decreased heart rate during immersed

exercise while breathing air at 6.76 ATA compared to 1 ATA.

4.4 Susceptibility Determination

Susceptibility to oxygen toxicity is widely variable, making definitive dose-response relationships difficult to formulate although some individuals exhibit unusual susceptibility. Variability in susceptibility exists between subjects and for a single subject from day to day . Attempts to correlate factors such as age, weight, physical fitness, smoking, alcohol ingestion, psychological stability or personality traits have failed (Donald, 1947).

We found variability in susceptibility to CNS oxygen toxicity between subjects and in the same subject in repeated exposures. In Phase 1, only 2 of the 7 subjects who repeated experiments consistently had symptoms or consistently lacked symptoms each time. The two subjects who consistently had symptoms reported the same symptoms each time. Another subject had symptoms each time (3 times, though not the same symptom) when tested with a hypoxic exposure but only once when tested without the hypoxic exposure (5 times). Overall, the presence of a hypoxic exposure did not significantly effect the incidence of symptoms. In Phase 2, neither of the 2 subjects who did repeated experiments had consistent symptoms, although one subject experienced facial twitching in both wet exposures and 1 of 2 dry exposures.

Susceptibility to the toxic effects of hyperbaric oxygen depends on the biochemical, physiological, and metabolic status of the CNS and oxygen delivery to the CNS. We measured ventilation and cerebral oxygenation in an attempt to correlate measured changes which influence oxygen delivery with CNS oxygen toxicity symptom incidence.

Ventilation is controlled by blood chemistry, neurological signals, and mechanical stimuli and effects blood O_2 and CO_2 content, the major determinants of CNS oxygen delivery. In Phase 1 experiments with symptoms, ventilatory response to CO_2 was reduced further at 2.80 ATA $P_{I_{O_2}}$ than in experiments without symptoms. Depressed ventilatory response to CO_2 increases cerebral $P_{t_{CO_2}}$, possibly leading to cerebral vasodilatation and cerebral

oxygenation which increases the risk of CNS oxygen toxicity. Susceptibility to CNS oxygen toxicity may be related to CO_2 retention as a result of depressed ventilatory response to CO_2 (Lanphier, 1975). In Phases 1 - 3, ventilatory response to CO_2 was depressed at 2.80 ATA PI_{O_2}. Depressed ventilatory response to CO_2 during hyperoxia is mediated by suppression of central mechanisms of respiratory control (Lambertsen, 1978). In Phase 3, the subject with the lowest ventilatory response to CO_2 had a convulsion, but the subject with one of the highest ventilatory responses to CO_2 had symptoms. In Phase 2, ventilatory response to CO_2 between groups with and without symptoms were not significantly different. Ventilatory response to CO_2 is variable between subjects and for the same subject on different days (Rebuck and Slusky, 1981). The apparent conflict in the significance of correlation of ventilatory response to CO_2 and symptom incidence between Phases 1 and 2 may reflect differences in position or duration of rebreathing (Phase 1 durations were shorter) or that variability of susceptibility is not directly reflected by ventilatory response to CO_2. In Phase 3, the subject who convulsed and the subject with symptoms had the lowest and highest ventilatory response to CO_2, respectively, at 0.21 ATA PI_{O_2}.

If a sufficient amount of oxygen is present at the cellular level in the CNS, the high oxidative metabolic rate will be surpassed, surplus oxygen will lead to oxygen radical formation, protective mechanisms will be overcome and cellular damage will result. If enough cellular changes take place, tissue malfunction will occur and symptoms of CNS oxygen toxicity become evident. In order to reach toxic levels, the oxygen content delivered to the CNS must overcome vascular regulation and the high metabolic rate of the CNS. We measured cerebral oxygenation changes using NIRS to investigate the correlation between oxygen delivery and CNS oxygen toxicity symptom incidence.

Local cerebral tPO_2 is determined by blood oxygen content, regional cerebral blood flow, and regional oxygen consumption. Blood oxygen content is controlled by inspired O_2 and CO_2 partial pressures, variables which were controlled or measured during this study. Lambertsen (1953) showed that oxygen consumption was not increased by hyperoxia.

Cerebral tBV changes have been directly correlated with cerebral blood flow (Colacino et al, 1981) and the redox state of cytochrome a,a_3 is an indicator of mitochondrial oxygen sufficiency.

In Phase 1, experiments showed vasoconstriction in hyperoxic exposures without inspired CO_2 and reversal of vasoconstriction with inspired CO_2, 60 torr $P_{ET}CO_2$. The degree of vasoconstriction reversal differed between experiments with definite symptoms and those without symptoms. Experiments with definite symptoms still had significantly reduced blood volume even at maximum CO_2 at 1.75 and 2.80 ATA PI_{O_2} relative to normoxia while experiments without symptoms showed no significant blood volume change from control even at 2.80 PI_{O_2}. Experiments with minor symptoms appeared to exhibit significant trends common to both other groups. Oxidation of cytochrome a,a_3 increased at both 1.75 and 2.80 ATA PI_{O_2}.

In Phase 2, a trend toward vasoconstriction was observed, though not statistically significant, in hyperoxic exposures without inspired CO_2 and reversal of vasoconstriction with inspired CO_2 at 60 torr $P_{ET}CO_2$. The degree of vasoconstriction reversal differed between experiments with definite symptoms and those without symptoms. Experiments with symptoms showed no change in tBV even at maximum CO_2 at 1.75 and 2.80 ATA PI_{O_2} relative to normoxia while in experiments without symptoms tBV increased at maximum CO_2 though not significantly at 2.80 ATA PI_{O_2}. Cerebral cytochrome a,a_3 oxidation was increased during hyperoxia and further increased at maximum CO_2. There were no significant differences in the amount of cerebral oxygenation between subjects with and without symptoms. Head-out immersion had no significant effect on symptom occurrence during Phase 2. In Phase 3, the subject who convulsed and the subject with symptoms had the largest increases in cytochrome oxidation at 0.21 ATA PI_{O_2} and 2 of the 3 largest increases in cyt. a,a_3 at 2.80 ATA PI_{O_2}.

Correlation of symptoms with ventilatory and cerebral oxygenation changes were not the same for all Phases in which symptoms occurred. This could be due to the differences in the

studies. Phases 1 and 2 were performed on subjects in different body positions, which effect ventilation and hemodynamics. Phase 3 was done using a lower inspired CO_2 and longer duration of CO_2 exposure.

One trend present in Phases 1 and 2, vasoconstriction, was maintained in subjects with symptoms even at maximum CO_2, is contrary to previous findings. CNS oxygen toxicity may be related to the ability of the cerebral vessels to maintain vasoconstriction (Torbati, 1987). Ischemic injury has been shown to diminish cerebral blood flow autoregulation leading to increased cerebral oxygenation (Miller et al, 1970). Ischemia secondary to vasoconstriction may lead to increased oxygen delivery to the brain by leading to increased $tPCO_2$ or decreased CO_2 buffering by hemoglobin (Lambertsen, 1955). Bean (1972) showed that the vasoconstriction due to hyperoxia fails prior to seizure onset and the initial decrease in cerebral blood flow is reversed with increasing duration.

Definite symptoms would be expected to occur during increased oxygen delivery according to the working hypothesis. Maintenance of oxygen induced vasoconstriction would tend to limit oxygen delivery. The results of the current study may be due to the fact that the brain is heterogeneous in terms of oxidative metabolism and blood flow (Hoshi et al, 1994; Bean, 1961). A range of P_{O_2}'s, 1-90 torr, have been found simultaneously in different areas of the brain (Lubbers and Starlinger, 1975). The area of the brain illuminated by the NIRS may not reflect changes that occur in other areas when an intervention is initiated or may show opposite effects from areas which are preferentially stimulated. Increases in blood volume may have occurred in other areas of the brain, where conditions of increased oxygenation may have precipitated toxicity and thus the appearance of symptoms. Alternatively, the reduced vasodilatory response may have been mediated by the development of oxygen toxicity. Increased formation of oxygen radicals attributed to causing oxygen toxicity may cause a vasoconstriction which overrides the effect of increased CO_2.

The predictive power of the techniques used in this study may be in their combination. The subject who convulsed had the lowest ventilatory response to CO_2 at 0.21 ATA PI_{O_2}

while simultaneously having the largest increase in cytochrome a,a_3 oxidation. One finding in this study which proved to be very reproducible and may correlate with symptoms at longer durations or higher PI_{O_2}'s was tBV increase and cyt. a,a_3 oxidation increase during facial immersion. These changes could be part of the human dive reflex and may contribute to CNS O_2 toxicity susceptibility during wet dives. These findings suggest that this susceptibility might be reduced if the diver's face was dry. Future studies should concentrate on the role of CO_2, facial immersion, and the use of logistic regression to correlate NIRS data with ventilatory response data.

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6. PUBLICATIONS

Natoli, M.J., R.D. Vann, R.E. Moon, and C.A. Piantadosi. 1991. Assessment of factors affecting CNS oxygen toxicity using near infrared spectroscopy and ventilatory response. Undersea Biomed. Res. 18(Suppl.): 88.

Natoli, M.J., R.D. Vann, R.E. Moon, and C.A. Piantadosi. 1993. Assessment of factors affecting CNS oxygen toxicity using near infrared spectroscopy. Undersea Biomed. Res. 20(Suppl.): 13-14.

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suppression and varying activity by junctional and ventricular pacemakers and variability in HR with rapid beat to beat changes from as low as 8.5 bpm to over 100 bpm. Thus, a very different picture from the classical smooth diving bradycardia was observed. Similarly, there was considerable variability in cardiac output with only a small reduction in subject A, a fall from about 7 L/min to 6 L/min in B and from 6 L to 2 L/min in C. At maximum depth all three subjects showed a pronounced peripheral vasocontriction resulting in cessation of blood flow and even a reduction of calf circumference indicative of a forceful capacitance vessel constriction. In subject A, the blood pressure was 250/145 mm Hg at max depth vs a control value of 150/80 mm Hg and in subject C, the values were 180/100 vs 155/70 respectively. These values at max depth, coinciding with low cardiac outputs, attest to the vasoconstriction which is thought to be the primary factor in the cardiovascular diving response. Supported by NOAA through NY Sea Grant, NA90AADSG517.

146. ASSESSMENT OF FACTORS AFFECTING CNS OXYGEN TOXICITY USING NEAR INFRARED SPECTROSCOPY AND VENTILATORY RESPONSE. M.J. Natoli, R.D. Vann, R.E. Moon, and C.A. Piantadosi. Hyperbaric Center, Duke University Medical Center, Durham, NC 27710.

Elevated partial pressures of inspired oxygen and CO_2 reduce the time to onset of symptoms of central nervous system (CNS) oxygen toxicity. The effects of hyperoxia and hypercapnia on cerebral oxygenation, ventilatory response, and symptoms were investigated in 11 subjects using a computer controlled, closed-circuit breathing apparatus to maintain PIO_2's of 0.21, 1.75 and 2.80 ATA. Minute ventilation was measured during rebreathing as the end-tidal CO_2 rose to 60 torr. Relative changes in cerebral oxygenation from normoxia were monitored using near infrared spectroscopy (NIRS), a non-invasive technique for continuous measurement of Hb, HbO_2, blood volume and cytochrome a,a_3 redox level. Definite symptoms (tunnel vision, ringing in the ears, and irritability) associated with CNS oxygen toxicity were reported within 3 torr of the maximum $PetCO_2$ at 2.80 ATA PIO_2 in 6 of 34 experiments. Another 15 minor symptoms (tingling, numbness, narcosis, sweats, and dizziness) were reported. All subjects with definite symptoms and 7 of 10 with minor symptoms showed low ventilatory response slopes (ventilation vs. $PetCO_2$) at 2.80 ATA PIO_2 relative to normoxic slopes. Cerebrocortical blood volume relative to normoxia, decreased in the overall group with increasing PIO_2. CO_2 rebreathing reversed this effect such that relative cortical blood volume was significantly greater ($p<.0001$) at a $PetCO_2$ of 60 torr than at normocapnia for all PIO_2's tested. Despite the blood volume response to CO_2, the amount of oxidized cytochrome a,a_3 did not change significantly, suggesting metabolic regulation of the mitochondrial redox state. Experiments in which symptoms were reported resulted in smaller blood volume changes at maximum $PetCO_2$ for a PIO_2 of 2.80 ATA than at 0.21 ATA PIO_2 (and a tendency for the amount of oxidized cytochrome to increase). In summary, ventilatory response measurements suggest some individuals have depressed CO_2 response during hyperoxia and may be more susceptible to CNS oxygen toxicity. The use of NIRS during hyperoxic hypercapnia shows evidence for an intact regulatory mechanism for the oxidation state of cytochrome a,a_3 in the respiratory chain and symptoms of CNS oxygen toxicity may be present without reversal of hyperoxic cerebral vasoconstriction during CO2 rebreathing. Supported by ONR contract No. N00014-88-0400.

147. ANALYSIS OF THE EFFECTS OF WEIGHT PLACEMENT ON THE ENERGY COST OF UNDERWATER SWIMMING. M. E. Tedesco and David R. Pendergast. Hermann Rahn Laboratory of Environmental Physiology, Department of Physiology, State University of New York at Buffalo, 124 Sherman Hall, Buffalo, New York 14214.

Divers routinely wear weights to overcome the buoyancy of their gear. Little attention has been given to the effect of the placement of these weights on the energy cost of swimming. This study determined the effect of the placement of weights (44 N) between the chest, waist, knee

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treatment (0.00013 rate). The 2.4 ATA group had three oxygen related complications during 5,268 treatments (0.00057 rate). Summing the two high pressure groups, there were 4 oxygen related seizures in 12,484 treatments, compared to zero in the low pressure group of 12,468 treatments. There was no mortality in any group, and the most common morbidity was a rare mild aural barotrauma. Outcomes in all groups were similar. Of note is that in this series of 24,952 treatments, there were no deaths, a seizure incidence rate of 0.00016 (0 at 2.0 ATA), and no significant adverse effects, once again showing the safety of HBOT (this represents 5.7 man-years in the chambers). We have begun the Phase II, low pressure end, of the study at 1.8 ATA.

3. RESULTS OF COMBINATION OF HYPERBARIC OXYGEN THERAPY WITH NALOXONE IN EXPERIMENTAL SPINAL CORD INJURY. MN Demircan, İ Öztek, ME Elbüken. Gülhane Military Medical Academy,Haydarpaşa Training Hospital;Neurosurgery,Pathology,Sea and Underwater Clinic,Kadıköy 81327,İstanbul.

BACKGROUND: Ischemia and hypoxia play a role on spinal cord injury. It has been suggested that Naloxone(NLX) can improve regional blood flow in the traumatic area of the spinal cord.On the other hand HBO therapy is reasonable for tissue hypoxia and its beneficial effects has been reported.
METHODS: Experimental cord traumas were performed on 30 rabbits.The animals were divided into three groups as control,HBO-therapy and HBO-NLX combination therapy.HBO was applied as 100% O_2 at 2 ATA pressure daily in one session for 2 hours in experimental pressure chamber for seven days.NLX was administered 0.4 mg/kg intravenously bolus 1/2 hour before HBO-therapy.After neurologic evaluation(the scale,described by Tarlow was used for neurologic evaluation on the day of trauma and the 8^{th} post-traumatic day)on the 8^{th} post-traumatic day,all animals were sacrificed and their spinal cord were removed for pathologic evaluation. Stromal and cellular changes were examined under light microscope.For statistical analysis one-way Anova test,Kruskal-Wallis variance analysis test and t-test were used.
RESULTS:In control group,improvement of neurological grade was observed in one animal ($F:0.130, P > 0.05$).In HBO-therapy group,improvement of neurological grade were observed in seven animals($F:7.515, P < 0.05$).In HBO-NLX therapy group,improvement of neurological grade were observed in six animals($F:3.115, P > 0.05$).As a result of pathological evaluation, there were statistically significant differences between the control and HBO-therapy,HBO-NLX combination therapy group($P < 0.05$).
CONCLUSION: HBO-therapy has positive effect on experimental spinal cord injury.We observed small beneficial effect of HBO-therapy neurologically and NLX administration did not improve this result.

4. **ASSESSMENT OF FACTORS AFFECTING CNS OXYGEN TOXICITY USING NEAR INFRARED SPECTROSCOPY.** MJ Natoli, RD Vann, RE Moon, and CA Piantadosi. FG Hall Hypo/Hyperbaric Center, Box 3823, Duke University Medical Center, Durham, NC 27710.

BACKGROUND: Elevated O_2 and CO_2 pressures and immersion are factors which speed the onset of symptoms of central nervous system oxygen toxicity (CNS O_2Tox).
METHODS: The effects of hyperoxia, CO_2, and head-out immersion on cerebral oxygenation were investigated in healthy volunteers and related to CNS O_2Tox symptoms. Inspired O_2 pressures (P_iO_2) of 0.21, 1.75, and 2.80 ATA were maintained by a computer controlled, closed-circuit breathing apparatus. The end-tidal CO_2 ($P_{et}CO_2$) either was allowed to rise from 40 to 60 torr (ramp) or held constant at 50 torr for 10 min (isocapnia). The ramp studies were conducted dry or immersed while the isocapnia studies were dry only. Changes in cerebral oxygenation and blood volume were monitored using near infrared spectroscopy (NIRS).
RESULTS: In 9 isocapnia studies, one convulsion occurred at 2.80 ATA O_2. In the ramp studies, symptoms associated with CNS O_2Tox were reported in 5 of 13 dry and 5 of 13 immersed experiments at 2.80 ATA O_2 and $P_{et}CO_2 > 57$ torr.

Mean NIRS Signal Changes Relative to Normocapnic Normoxia

	Blood Volume			Cytochrome Oxidation		
P_iO_2 (ATA)	0.21	1.75	2.80	0.21	1.75	2.80
†Normocapnia	0.0	-.007±.09	-.026±.10	0.0	+.158±.19#	+.126±.17#
†$P_{et}CO_2$=60torr	+.024±.04*	+.011±.10*	-.025±.10#	+.154±.09*	+.315±.24*#	+.260±.22*#
‡$P_{et}CO_2$=50torr	+.062±.04*	+.011±.06	+.018±.06	+.194±.11*	+.258±.23*	+.266±.13*

* Sig. diff. from normocapnia, $p < 0.05$, # Sig. diff. from normoxia $p < 0.05$, † n=26, ‡ n=9.

CONCLUSION: Cytochrome oxidation increased with increasing $P_{et}CO_2$ and P_iO_2. Hyperoxic vasoconstriction is reversed by CO_2 at P_iO_2's ≤ 1.75 ATA O_2 but not at 2.80 ATA O_2 and not in subjects with CNS O_2Tox symptoms. Head-out immersion had no significant effect on cerebral oxygenation compared to dry exposure. The largest increase in oxidized cytochrome a,a_3 at 0.21 and 2.80 ATA O_2 was found in the subject who convulsed. Therefore, monitoring by NIRS may be predictive of susceptibility to CNS O_2Tox. Supported by ONR Contract No. N00014-88-0400.

5. TRANSCRANIAL DOPPLER EVALUATION OF CEREBRAL VESSELS DURING HYPERBARIC OXYGENATION. *CE Fife, *S. Hanson, !JS Meyer, *N Maklad. *Departments of Anesthesiology, Neurology and Radiology, University of Texas Medical School, Houston, TX 77030 and !Baylor College of Medicine Cerebral Vascular Research Laboratory, Department of Veteran's Affairs Medical Center, Houston, TX 77030.

BACKGROUND/METHODS:Randomized blinded data suggest HBO at 2ATA relieves acute migraine pain in 70% of patients, presumably due to hyperoxic vasoconstriction. Transcranial Doppler (TCD) examinations were carried out on normal volunteers and pain free migraineurs to determine whether this technology was sufficiently sensitive to detect flow velocity changes which may result from oxygen administration. Middle cerebral artery (MCA) signals were monitored while subjects respired air at 1 ATA, 10% O_2 at 2ATA and 100% O_2 at 2ATA via Scott face masks. Mean flow velocity (MFV), pulsatility index (PI), blood pressure and pulse were recorded at 10 min intervals. RESULTS: Seven pain free female migraineurs and 10 controls of either sex were age matched. No change was noted in MCA MFV and PI between baseline (room air) and 10% O_2 at 2ATA ($p=.985$, $p=.660$). Upon hyperbaric oxygen exposure, all subjects demonstrated a statistically significant decrease in MCA MFV ($p=<0.001$). No differences were noted between the migraineur and non-migraineur groups. MCA MFV returned to baseline values almost immediately upon surfacing. CONCLUSION: TCD evaluation is technically possible in the hyperbaric environment with customized equipment, and sufficiently sensitive to detect flow velocity changes with oxygen breathing. There is a significant decrease in MCA mean flow velocity during respiration of hyperbaric oxygen compared to normoxic control conditions. These preliminary results are compatable with distal vasoconstriction as expected. Further TCD studies throughout a range of oxygen concentrations are warranted. Supported by National Headache Foundation Grant #HSC-MS-90-093.

6. CHRONIC CO EXPOSURE INDUCES STRESS PROTEIN 72 AND SUPPRESSES IL-1ß PRODUCTION IN MACROPHAGES AFTER ENDOTOXIN STIMULATION *IN VITRO*. S.D. Brown, J. Peré, A. Holian. University of Texas Health Sciences Center and Hermann Center for Hyperbaric Medicine, Houston, TX 77030.

Introduction: CO directly stimulates soluble guanylate cyclase and thus mimics the effect of nitric oxide (NO) on guanylate cyclase to increase intracellular guanosine 3',5'-cyclic phosphate (c-GMP). Unlike NO, CO is not rapidly metabolized and chronic stimulation of that enzyme by CO may have different effects than NO. Ordinarily, increased c-GMP alters the activities of many proteins via stimulation of protein kinases and alterations of intracellular calcium. Human alveolar macrophages (HAM) are important in surveillance for infection and early tumors and are exposed to high levels of CO in smokers. Stress proteins (SP) are elaborated by HAM in response to a variety of insults and may be mediated in part by cyclic nucleotides. The stress response suppresses a number of cellular functions including Il-1ß production. Thus, CO may affect the immune function of these cells. **Methods:** HAM from volunteers were washed and resuspended in appropriate media. HAM were incubated in either room air (RA), 1% CO (balance air) or 7% O_2 (balance N_2)

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were 46.7 mm Hg at the shallow depth and 52.4 mm Hg at the great depth but were unaffected by the R/E combination. $\dot{V}_E$ was not affected differently by the R/E combinations, but the tidal volumes were reduced 8-20% by increased E. The ERV was reduced by about 20% with the higher E. Similarly, the VC was reduced by about 15%. The T_i/T_{tot} was not affected by the R/E combinations but it increased from 0.45 to 0.47 with depth. The dyspnea scores were not different for the different loads. Conclusion: R and E are additive in their effects on divers' respiratory performance. Supported by US Naval Medical R&D Command Contract N000149310509.

153. **MIDDLE EAR O_2 AND CO_2 TENSIONS AND THE EUSTACHIAN TUBE PRESSURE EQUALIZATION ABILITY.** **A Shupak (1,2), JD Swarts (2), CD Bluestone (2), WJ Doyle (2). Israel Naval Medical Institute, Haifa, Israel (1) and Dept. of Pediatric Otolaryngology, Children's Hospital of Pittsburgh (2).**

BACKGROUND: Middle ear barotrauma related to Eustachian tube (ET) malfunction is the most frequent diving-related disorder. Changes in middle ear oxygen and carbon dioxide partial pressures occur during diving secondary to the increased ambient pressure, use of oxygen-enriched breathing mixtures, and carbon dioxide retention. The purpose of the present study was to learn to what extent such changes potentiate or interfere with the ET ventilatory function (ETVF). METHODS: ETVF was investigated in four young adult female Cynomolgus monkeys by the Forced Response and Inflation-Deflation tests, using the following gas mixtures: 1) Air. 2) Hypoxic mixture: 12% O_2, 88% N_2. 3) Hyperoxic mixture: 100% O_2. 4) Hypercarbic mixture: 5% CO_2, 21% O_2, 74% N_2. RESULTS: Lower opening and steady state pressures and passive resistance were observed under middle ear hypercarbic and hypoxic conditions ($0.05 < p < 0.1$, repeated measures ANOVA). The variances in these parameters were all explained by the differences between the hyperoxic and hypercarbic conditions. For the inflation-deflation test, the maximal pressure change on a single swallow, the mean pressure drop per swallow, and the percentage of the applied pressure equalized, were all found to be greater for the inflation test under hypoxic and hypercarbic conditions ($0.05 < p < 0.1$, repeated measures ANOVA and Kruskal-Wallis non-parametric ANOVA by ranks). CONCLUSION: The results show that middle ear O_2 and CO_2 tensions have a regulatory role with respect to ETVF. This observation adds to our understanding of the pathophysiology of middle ear barotrauma associated with diving, and might provide an explanation for the high incidence of negative middle ear pressures and effusions reported after oxygen diving.

154. THE EFFECT OF THE HUMAN DIVE REFLEX ON CEREBRAL OXYGENATION. MJ Natoli, RD Vann, RE Moon, and CA Piantadosi. FG Hall Hypo/Hyperbaric Center, Box 3823, Duke University Medical Center, Durham, NC 27710.

BACKGROUND: Human responses to facial immersion and apnea, the dive reflex, include bradycardia and peripheral vasoconstriction. These responses are thought to be part of an adaptive oxygen conservation reflex which redistributes blood flow to the brain and heart. This abstract quantifies the effect of the diving reflex on the brain.

METHODS: Cerebral oxygenation, heart rate, inspired O_2 and end tidal CO_2 were monitored in 6 healthy subjects immersed to the neck and over the face in thermoneutral water (35° C). Cerebral oxygenation, oxidation of cytochrome a,a_3 and changes in tissue Hb, HbO_2, and blood volume, was monitored using near infrared spectroscopy. Subjects breathed air from a rebreather for 10

min under each of the following conditions: (1) head-out (HO), (2) fully immersed (Im), (3) head-out recovery (REC), and (4) fully immersed. One minute breath holds were performed during the first head-out period (BH+HO) and the second full immersion period (BH+Im).

RESULTS: Facial immersion increased cerebral oxygenation by increasing $tHbO_2$, tBV, and cytochrome a,a_3 oxidation. Apnea further increased cerebral oxygenation during facial immersion but not during head-out immersion. Significant bradycardia occurred during breath holding but not during facial immersion. PET_{CO_2} was unchanged. Results are shown in the table (mean ± SD):

	HO	BH+HO	Im	REC	BH+Im
Δ Cyt. a,a_3 †	0	2.0±1.7	7.8±6.8*	-3.3±7.0*	14.1±9.0•‡
Δ tBV †	0	1.3±1.3	6.2±1.9*	-4.0±3.7*	10.0±8.3•‡
Δ tHb †	0	0.1±1.3	-3.4±2.5*	-0.1±2.6*	-2.1±3.4
Δ $tHbO_2$ †	0	1.2±1.2	9.9±8.8*	-3.8±5.2*	12.1±9.6•‡
HR (bpm)	72.0±10.5	61.0±5.8*	75.5±10.4	68.8±9.0	67.5±9.9‡

† Δ optical density x 100, changes relative to HO ‡ Sig. diff. from full immersion, $p < 0.05$
* Sig. diff. from preceding condition, $p < 0.05$ • Sig. diff. from BH+HO, $p < 0.05$

CONCLUSION: The increase in cerebral blood volume and oxidation state due to facial immersion may contribute to CNS O_2 toxicity susceptibility during wet dives. These findings suggest that this susceptibility might be reduced if the diver's face was dry. Supported by ONR N00014-88-0400.

155. A PROGRESS REPORT ON THE PROSPECTIVE RANDOMISED DOUBLE-BLIND CONTROLLED STUDY OF OXYGEN AND OXYGEN-HELIUM IN THE TREATMENT OF AIR-DIVING DECOMPRESSION ILLNESS (DCI). A Drewry, DF Gorman. Diving and Hyperbaric Unit, RNZN Hospital, Naval Base, Auckland, New Zealand.

BACKGROUND: The study was initiated because of the high failure rate in treating recreational divers with DCI using the USN recompression algorithms. Studies of air bubbles in animals and case reports of treatment success in humans, support the assertion that there may be advantages for using oxygen-helium over oxygen in the treatment of DCI.

METHODS: All recreational divers with symptoms of DCI after air-diving are being evaluated and entered into the study. Patients are randomly allocated to receive either 50/50 oxygen-helium or 100% oxygen and compressed to 2.8 Bars abs. Treatment depth, duration and frequency is determined by symptom response. Patients are evaluated by medical examination and psychometric testing after the first treatment, before discharge, after one month and at one year.

RESULTS: The study has been in progress for 2 years and to date, 88 subjects have been entered, of whom 18 failed to meet the trial criteria (due to pregnancy, wrong diagnosis or failure to follow the study protocol). The one-year follow-up results for 56 subjects are available. The treatment groups are directly comparable with respect to subject age, sex and symptoms but there is a statistically significant difference ($p=0.03$) in the requirement for multiple recompressions; 20 of 31 in the oxygen group did not have a sustained recovery after the first recompression compared with 9 of 25 in the oxygen-helium group. The trial is continuing.

APPENDIX A :

Duke Institutional Review Board Protocol and Modifications

This appendix contains the Informed Consent documents approved by the Duke University Medical Center Institutional Review Board and signed by each subject prior to each experiment. The appendix also contains a letter requesting modifications and the resultant modified Informed Consent.

INFORMED CONSENT FORM - PART I

Clinical Investigation

Consent to Participate in Research Study

IRB # 587-87-8R1
PROTOCOL TITLE: Factors Affecting CNS Oxygen Toxicity in Humans.

The chambers at the F. G. Hall Laboratory can be used to simulate various depths beneath the seas by pumping air into the chambers and thus increasing atmospheric pressure. They can also be used to simulate various heights above the earth by sucking air out of the chambers and thus decreasing atmospheric pressure. Exposure to such changed atmospheric pressures will involve changes in pressure both inside and outside the body. The potential hazards of such exposures may be outlined as follows:

1. Hazards associated with compression or increasing the air pressure inside the chambers (simulation of depths beneath the sea or rapid return to the surface from simulated altitude).With compression there is occasional difficulty getting the air pressure in the ears, sinuses, teeth, lungs and intestines to equal the increasing pressure outside the body. Such problems may cause pain and the production of fluid in these spaces. Hearing loss, inflammation of the ear and sinusitis may occur. Usually, these problems are temporary and clear in a few days. Very rarely permanent problems occur. However, if any discomfort is felt during compression, the personnel in the laboratory should be immediately notified so that corrective measures can be taken. Occasionally individuals have air filled cysts in their lungs. If such a person is exposed to increased pressure, the cyst could possibly rupture and cause the lung to collapse, requiring medical and/or surgical treatment such as inserting a tube through the skin into the chest to re-expand the collapsed lung. This complication is rare and, thus far has not occurred in this laboratory in greater than 20 years of experience involving thousands of patient exposures.

2. Risks associated with decreases in air pressure or decompression from simulated depths beneath the sea:

   With decrease in air pressure such as is encountered during decompression from simulated depths, or exposure to simulated altitudes, symptoms such as joint pain can occur and are termed decompression sickness. The cause is thought to be the formation of gas bubbles in the body. These bubbles can cause damage to the brain, spinal cord, death and disability. In this laboratory, the depth as well as the rate of changes in pressure are carefully controlled, and only mild and transient forms of decompression sickness have been seen here, and these occur in less than 1% of dives.

   Early symptoms of bubbles may be pain in the joints, skin rash or, if an individual has had migraines in the past, a migraine headache. If these symptoms occur, either during the test or (and this is important) at any time after the test, the Hall Laboratory personnel should be notified immediately by calling (919) 684-8111 and asking for the hyperbaric physician on call, for many, but not all, cases of decompression sickness can be cured by prompt recompression.

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3. Other hazards associated with exposures to increased atmospheric pressures (simulated depths beneath the sea):

   Exposure to higher than normal oxygen concentrations can cause generalized shaking and even seizures. A potentially hazardous exposure to an increased amount of oxygen in part of the study could occur if treatment of decompression sickness is necessary.

   An additional potential hazard associated with exposure to increased atmospheric pressure is destruction of certain parts of long bones. Experts generally agree that this problem is exceedingly rare with exposures to simulated depths of less than 100 feet and/or for exposure times not exceeding three to four hours.

4. Risks associated with exposure to simulated altitudes (sucking air out of the chambers):

   Individuals exposed to simulated altitudes, decreased atmospheric pressures, can become unconscious and seriously harmed if the amount of oxygen available for breathing becomes too low. If a test exposure to a less than normal amount of oxygen is part of the treatment design, the physician reviews thoroughly beforehand with the subject and details and risks of the experiment. The details and risks are contained in Part I. If an individual during an exposure to simulated altitude feels lightheaded or notices any discomfort or unusual sensations, he should notify the chamber personnel immediately. Also decompression sickness as noted above can occur with altitude exposures. This complication is unusual. If an individual develops signs or symptoms of low oxygen or decompression sickness during altitude exposure, the chamber might have to be recompressed or returned to the surface rapidly. This rapid increase in chamber pressure would expose individuals to increased risks of equalizing air pressure to the ears, sinuses, teeth, intestines or lungs with possible injury to these structures as noted above in the discussion of hazards of compression or increase in the air pressure inside the chambers. These complications are unusual.

5. Risks associated with equipment failure:

   If there is mechanical or electrical failure of part of the pressure tank or of equipment which keeps it operating safely, the exposed humans could be seriously injured or even killed. If a fire occurs within the pressure tank, all exposed humans could be burned or asphyxiated. In over twenty years of operation, there have not been any instances of structural failure or fire in the Duke chambers. Moreover, all new equipment is subjected to evaluation and testing before its use is permitted in the chamber. A regular preventive

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maintenance program is utilized for all systems. Nonetheless, the possibility of equipment failure, however remote, cannot be completely eliminated.

6. General risks:

   It is important that individuals about to undergo tests involving the changing of atmospheric pressures understand that many of these tests have never been performed under such conditions before and there may be risks which are unknown. If all was known about what happened or what might happen, there would be no reason to do the tests.

   Physicians have been instructed to answer any questions concerning risks and safety measures. Individuals about to undergo such tests should also understand that the test should not be done if their consent has been effected by a promise of a large sum of money or by other pressures to participate.

7. Provision of care:

   Immediate necessary care is available if an individual is injured because of participation in this treatment. However, there is no provision for free medical care or for monetary compensation for such injury. Further information can be obtained from the Hospital Risk Management Office at 684-3277.

8. Photography:

   I hereby give permission to Duke University Hospital to make any photographs for diagnostic purposes and/or to enhance the medical record. I further authorize the use of such photographs for teaching purposes or to illustrate scientific papers or lectures without inspection or approval on my part of the finished product or the specific use to which it may be applied.

9. Pregnancy statement:

   There is evidence to support that the frequency of birth defects is significantly greater among children from pregnancies during which women have been exposed to increased pressures as for example in diving.

   It is therefore necessary that a pregnancy test be done first on women of childbearing potential. To my knowledge I am not pregnant at the present time. Further, if sexually active, I will take contraceptive precautions for the duration of this treatment.

10. Statement by subject:

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Initials

I have read Parts I and II of this Informed Consent and have been given the opportunity to discuss this treatment and to ask questions. I have been informed that I may contact Richard Vann (Physician) at 684 - 3305 (telephone #) to answer any questions I may have during this treatment. I agree to participate as a patient with the understanding that I may withdraw at any time except for the necessity of staying in the chamber for the time required for decompression.

Signature _____ Date _____

Witness _____

INFORMED CONSENT FORM - PART II

Clinical Investigation

Consent to Participate in Research Study

IRB # 587-87-8R1
PROTOCOL TITLE: Factors Affecting CNS Oxygen Toxicity in Humans.

I
The purpose of this study is to measure a number of factors which might relate to the propensity to develop oxygen toxicity. A certain proportion of people may develop toxic symptoms when exposed to high oxygen concentrations under hyperbaric conditions. Symptoms may consist of nausea, twitching of their facial muscles or even frank epileptic seizures. The factors which determine the susceptibility of an individual to this condition are not entirely understood.

During the study you will be exposed to a number of condition, both while resting and moderately exercising. These conditions are : breathing air, oxygen, either of the first two gases with 3% carbon dioxide, immersed in water up to the neck or sitting in the dry. All possible combinations of the above factors will be experienced both at atmospheric pressure and at simulated pressure of 25 feet of seawater (fsw) (1.76 time the ambient atmospheric pressure). During each experimental period, which will consist of 10-15 minutes of either rest or exercise, several measurements will be made. First, an infrared light beam will be shined on your head. The small amount of infrared light that will be emitted back from the brain will allow us to assess the oxygenation of your brain cells. It will also allow us to monitor the blood volume in your head ant the amount of oxygen bound to blood hemoglobin. Periodically, small quantities of green dye (indocyanine green) will be injected into a vein. The infrared light technique will allow us to measure the total amount of blood flow to your brain. We will also measure expired gas oxygen and carbon dioxide concentrations, heart rate, respiration rate, and blood pressure. During one of the days of the study a small plastic catheter will be inserted under local anesthetic into one of the arteries in your wrist. This will allow us to collect blood for the purpose of measuring actual oxygen and carbon dioxide concentrations.

The general risks of hyperbaric exposure have been explained to you in Part I of the consent form. Additional risks of this study are:

(1) the possibility of an epileptic seizure due to the exposure to hyperbaric oxygen.
(2) the risks of arterial and venous catheters.
(3) the risk of exposure to infrared light
(4) the risk of injection of indocyanine green

There is a possibility that during exercise while breathing oxygen that you may have an epileptic seizure. You may already have experienced this during your diving career. the risk is probably increased during the time you are exercising and also further increased by breathing carbon dioxide. The risk of an epileptic seizure includes the risk of injury to yourself (for example, biting your tongue) and the attendant risk to your cardiovascular system of the increased metabolic activity and

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heart rate. Impaired brain function or death might even be conceivable; however, such is considered very unlikely from oxygen induced seizures in a chamber. It is possible, of course to drown while experiencing an epileptic seizure during diving. However, we will significantly reduce this risk by performing the experiment with your head out of the water. Furthermore, you will be suspended in a safety harness during these studies so that if you did experience a seizure your head would no actually fall into the water. You will be accompanied by a tender who has been trained to deal with the occurrence of oxygen convulsions.

The risk of intravenous and arterial catheter placement is related to the pin of the insertion (although local anesthetic will be used which should minimize this), infection, fainting, or even death due to an allergic reaction to the local anesthetic. A risk of occluding the artery leading to impaired hand function also exists. No complication of this sort have been experienced in the hyperbaric laboratory over a period of nearly 20 yrs. We will withdraw a total of about 200 ml (7 oz of about 1/2 pint) of blood during the course of this experiment. Before the study, the amount of your red blood cells will be checked. If this is normal and you have not recently donated blood the risk to you of withdrawing this quantity of blood is minimal.

During this study you will intermittently be exposed to infrared light rays at very low levels through the head. The amount of infrared light that your head will be exposed to is approximately equal to the amount that your experience while standing outside on a sunny day. The levels of light energy are between 500 and 10,000 times less than the existing exposure standard. As far as we know there are no ill effects from acute exposures of his sort.

An injection of green dye into a vein is routinely performed in this institution in order to measure the output of the heart. The risk is minimal. The dye has no toxic effect except that on rare occasions it may be associated with an allergic response. This effect is extremely rare, although theoretically it could cause death.

This study may have an adverse effect on an unborn child. Therefore, women of child-bearing potential must have a pregnancy test as indicated in Part I of this Consent Form.

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Initials

INFORMED CONSENT FORM - PART II

Clinical Investigation

Consent to Participate in Research Study

IRB # 587-87-8R1
PROTOCOL TITLE: Factors Affecting CNS Oxygen Toxicity in Humans.

I have read, understood, and have signed Part I of this Consent form. I have also read Part II and have been given the opportunity to discuss it and to ask questions. I agree to participate as a subject with the understanding that I may withdraw at any time.

Subject	Date	Witness	Date

Initials

J.S. Harris, M.D.
Box 3916
Duke University Medical Center

Dear Dr. Harris:

We would like to modify our experimental protocol (IRB No. 813-90-8R4), "Factors affecting CNS oxygen toxicity in humans." A description of these modifications is given below. The new studies have been proposed to the Navy and would be conducted over the next two years. The Informed Consent has been completely revised and is enclosed. The compensation for each phase of the study is indicated on the Informed Consent.

Central nervous system oxygen toxicity is a medical problem in hyperbaric oxygen therapy and an operational problem for divers. The Navy's interest is in both these areas. We believe that progress towards a solution is possible through understanding the underlying physiology. Our approach is to measure key physiological parameters (ventilatory response to carbon dioxide; cerebral oxygenation, blood flow and metabolism) under environmental perturbations which make small increases in the risk of oxygen toxicity.

Over the past two years, we have conducted 14 oxygen exposures at 1.75 ATM and 34 exposures at 1.75 and 2.8 ATM. In 21 of the 34 exposures at 2.8 ATM, there were symptoms consistent with mild oxygen toxicity as indicated below. There were no symptoms at 1.75 ATM. These symptoms were present only during carbon dioxide rebreathing at 55-60 torr end-tidal partial pressure and disappeared immediately upon cessation of rebreathing.

Number	Symptom
8	Sweating
7	Narcosis
5	Numbness
3	Feeling of imminent blackout
2	Dizziness
2	Floating feeling
2	Irritability
2	Tunnel vision
1	Change in depth perception
1	Tingling
1	Ringing in ears
1	Headache
1	Anxiety

Our previous studies are designated Phase (a) and are described below. The additions to these basic studies are designated Phase (b), etc.

(a) Subjects were exposed for 10-15 minutes to each of the following oxygen partial pressures: 0.11, 0.21, 2.8, and 1.75 ATM. The 0.11 ATM exposure terminated when

the subject's hemoglobin saturation fell to 70%. Ventilatory response to rebreathed carbon dioxide was measured at each partial pressure except for 0.11 ATM. The subjects were dry and at rest. Changes in brain cortex oxygenation, metabolism, and blood volume were measured using near-infrared (NIR) spectroscopy.

(b) The Phase (a) studies will be repeated with resting subjects immersed to the neck in 35°C (thermoneutral) water.

(c) Dry, resting subjects will rebreathe carbon dioxide at a constant end-tidal partial pressure of 50 torr for 5 minutes. (The normal end-tidal CO2 is 40-45 torr.) This differs from the previous studies in which the end-tidal CO2 rose from 45 to 60 torr over a 10-12 minute period. As before, the oxygen partial pressures will be 1.75 and 2.8 ATM.

(d) The effects of oxygen exposure duration will be studied at 1.75 and 2.8 ATM with no carbon dioxide in the inspired gas. The exposure at 2.8 ATM will be 20 min which is the standard exposure given routinely during the U.S. Navy Oxygen Treatment Tables 5 and 6. The exposure at 1.75 ATM will be 40 min which is within the equivalent U.S. Navy oxygen exposure limit of 75 min at 25 fsw (feet of sea water). To avoid possible interactions between lengthy hyperoxic exposures (and long decompression obligations) in a given experiment, the 1.75 ATM exposure will be conducted in the morning and the 2.82 ATM exposure in the afternoon. There will be two experimental days per subject. The subjects will be dry on the first day and immersed to the neck in 35}o{C water on the second day. Decompression, when required, will be conducted according to the procedures used in the past (enclosed) or with procedures computed by the same algorithm.

(e) The effects of oxygen exposure duration and work will be studied at 1.75 and 2.8 ATM. The subjects will pedal an ergometer while in a dry chamber or while immersed to the neck in water. The workload will be such as to produce an oxygen consumption of 1.5 lpm, and the exposure durations will be 40 min at 1.75 ATM and 10 min at 2.8 ATM. This is greater than the deepest allowed exposure in the U.S. Navy oxygen exposure limits for working operational divers (10 minutes at 50 fsw or 2.52 ATM).

During the studies of Phase (c) and the dry phase of (d), we will measure cerebral blood flow by intravenous injection of indocyanine green dye at 0.21 ATM (sea level) and before and during 50 torr end-tidal CO2 exposure at 2.82 ATM (85% oxygen at 77 fsw) and 1.75 ATM (85% oxygen at 35 fsw). A 5 ml bolus of sterile normal saline containing 0.5-1.0 mg/ml indocyanine green dye will be injected in 1 sec into a flexible indwelling catheter placed in an antecubital vein. The dye dilution curves will be measured by non-invasive, near-infrared (NIR) spectroscopy detectors placed over the forehead and carotid artery. There will be no more than six cardiogreen dye injections during a 24 hour period. Cardiogreen dye is used clinically to detect intracardiac shunts. Allergic reactions have been reported but are rare, and it has a half-life of 2-4 minutes.

The subjects will be briefed on the symptoms of oxygen toxicity and told to switch from oxygen to air should uncomfortable or disconcerting symptoms occur. They also will be told that convulsions can occur without prior warning and can occur shortly after a return to air breathing (the "off-effect"). A tender from the staff of the

Hyperbaric Center will accompany the subject, watch him closely for signs of toxicity, and switch him to air if any are observed. During dry, resting exposures, the subject will be in a semi-recumbent position in a lawn-chair. When not at rest in the chair (during immersion or dry or wet exercise), the subject will wear a safety helmet and will be supported by a safety harness to prevent injury in the event of a hyperoxic convulsion. When the venous catheter is in place, the subject's arm will be splinted and wrapped to protect it in the event of a convulsion.

The most significant manifestation of central nervous system oxygen toxicity is a convulsion. Oxygen convulsions are not harmful, and there have been no cases of neurological damage from uncomplicated episodes despite many which have occurred. Complications can occur as a result of aspiration or by striking hard objects. We will guard against these possibilities by use of a safety helmet and harness, by padding hard protrusions in the chamber, and by allowing neither food nor drink for two hours before a study. During the tonic phase of a convulsion, the subject becomes apneic and his tongue can be bitten, or it can occlude his airway. The tenders know to wait for the convulsion to subside and to check the airway to ensure it is clear. One of the F.G. Hall Hyperbaric Center diving physicians will be present during all studies. ;.pg; As before, subjects will be volunteers from the local community and will be compensated $75-100 per study. We still hope that Navy divers may be available as subjects, but this is uncertain at present. Subjects will will receive a physical examination by a Hyperbaric Center diving physician before acceptance into the study.

Sincerely,
R.D. Vann, Ph.D.

INFORMED CONSENT FORM - PART I

Clinical Investigation

Consent to Participate in Research Study

IRB # 813-90-8R4
PROTOCOL TITLE: Factors Affecting CNS Oxygen Toxicity in Humans.

The chambers at the F. G. Hall Laboratory can be used to simulate various depths beneath the seas by pumping air into the chambers and thus increasing atmospheric pressure. They can also be used to simulate various heights above the earth by sucking air out of the chambers and thus decreasing atmospheric pressure. Exposure to such changed atmospheric pressures will involve changes in pressure both inside and outside the body. The potential hazards of such exposures may be outlined as follows:

1. Hazards associated with compression or increasing the air pressure inside the chambers (simulation of depths beneath the sea or rapid return to the surface from simulated altitude).With compression there is occasional difficulty getting the air pressure in the ears, sinuses, teeth, lungs and intestines to equal the increasing pressure outside the body. Such problems may cause pain and the production of fluid in these spaces. Hearing loss, inflammation of the ear and sinusitis may occur. Usually, these problems are temporary and clear in a few days. Very rarely permanent problems occur. However, if any discomfort is felt during compression, the personnel in the laboratory should be immediately notified so that corrective measures can be taken. Occasionally individuals have air filled cysts in their lungs. If such a person is exposed to increased pressure, the cyst could possibly rupture and cause the lung to collapse, requiring medical and/or surgical treatment such as inserting a tube through the skin into the chest to re-expand the collapsed lung. This complication is rare and, thus far has not occurred in this laboratory in greater than 20 years of experience involving thousands of patient exposures.

2. Risks associated with decreases in air pressure or decompression from simulated depths beneath the sea:

   With decrease in air pressure such as is encountered during decompression from simulated depths, or exposure to simulated altitudes, symptoms such as joint pain can occur and are termed decompression sickness. The cause is thought to be the formation of gas bubbles in the body. These bubbles can cause damage to the brain, spinal cord, death and disability. In this laboratory, the depth as well as the rate of changes in pressure are carefully controlled, and only mild and transient forms of decompression sickness have been seen here, and these occur in less than 1% of dives.

   Early symptoms of bubbles may be pain in the joints, skin rash or, if an individual has had migraines in the past, a migraine headache. If these symptoms occur, either during the test or (and this is important) at any time after the test, the Hall Laboratory personnel should be notified immediately by calling (919) 684-8111

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Initials

and asking for the hyperbaric physician on call, for many, but not all, cases of decompression sickness can be cured by prompt recompression.

3. Other hazards associated with exposures to increased atmospheric pressures (simulated depths beneath the sea):

   Exposure to higher than normal oxygen concentrations can cause generalized shaking and even seizures. A potentially hazardous exposure to an increased amount of oxygen in part of the study could occur if treatment of decompression sickness is necessary.

   An additional potential hazard associated with exposure to increased atmospheric pressure is destruction of certain parts of long bones. Experts generally agree that this problem is exceedingly rare with exposures to simulated depths of less than 100 feet and/or for exposure times not exceeding three to four hours.

4. Risks associated with exposure to simulated altitudes (sucking air out of the chambers):

   Individuals exposed to simulated altitudes, decreased atmospheric pressures, can become unconscious and seriously harmed if the amount of oxygen available for breathing becomes too low. If a test exposure to a less than normal amount of oxygen is part of the treatment design, the physician reviews thoroughly beforehand with the subject and details and risks of the experiment. The details and risks are contained in Part I. If an individual during an exposure to simulated altitude feels lightheaded or notices any discomfort or unusual sensations, he should notify the chamber personnel immediately. Also decompression sickness as noted above can occur with altitude exposures. This complication is unusual. If an individual develops signs or symptoms of low oxygen or decompression sickness during altitude exposure, the chamber might have to be recompressed or returned to the surface rapidly. This rapid increase in chamber pressure would expose individuals to increased risks of equalizing air pressure to the ears, sinuses, teeth, intestines or lungs with possible injury to these structures as noted above in the discussion of hazards of compression or increase in the air pressure inside the chambers. These complications are unusual.

5. Risks associated with equipment failure:

   If there is mechanical or electrical failure of part of the pressure tank or of equipment which keeps it operating safely, the exposed humans could be

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seriously injured or even killed. If a fire occurs within the pressure tank, all exposed humans could be burned or asphyxiated. In over twenty years of operation, there have not been any instances of structural failure or fire in the Duke chambers. Moreover, all new equipment is subjected to evaluation and testing before its use is permitted in the chamber. A regular preventive maintenance program is utilized for all systems. Nonetheless, the possibility of equipment failure, however remote, cannot be completely eliminated.

6. General risks:

   It is important that individuals about to undergo tests involving the changing of atmospheric pressures understand that many of these tests have never been performed under such conditions before and there may be risks which are unknown. If all was known about what happened or what might happen, there would be no reason to do the tests.

   Physicians have been instructed to answer any questions concerning risks and safety measures. Individuals about to undergo such tests should also understand that the test should not be done if their consent has been effected by a promise of a large sum of money or by other pressures to participate.

7. Provision of care:

   Immediate necessary care is available if an individual is injured because of participation in this treatment. However, there is no provision for free medical care or for monetary compensation for such injury. Further information can be obtained from the Hospital Risk Management Office at 684-3277.

8. Photography:

   I hereby give permission to Duke University Hospital to make any photographs for diagnostic purposes and/or to enhance the medical record. I further authorize the use of such photographs for teaching purposes or to illustrate scientific papers or lectures without inspection or approval on my part of the finished product or the specific use to which it may be applied.

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Initials

Clinical Investigation

Consent to Participate in Research Study

IRB # 813-90-8R4
PROTOCOL TITLE: Factors Affecting CNS Oxygen Toxicity in Humans.

9. Pregnancy statement:

   There is evidence to support that the frequency of birth defects is significantly greater among children from pregnancies during which women have been exposed to increased pressures as for example in diving.

   It is therefore necessary that a pregnancy test be done first on women of childbearing potential. To my knowledge I am not pregnant at the present time. Further, if sexually active, I will take contraceptive precautions for the duration of this treatment.

10. Statement by subject:
    I have read Parts I and II of this Informed Consent and have been given the opportunity to discuss this treatment and to ask questions. I have been informed that I may contact Richard Vann (Physician) at 684 - 3305 (telephone #) to answer any questions I may have during this treatment. I agree to participate as a patient with the understanding that I may withdraw at any time except for the necessity of staying in the chamber for the time required for decompression.

_____ _____
Signature Date

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Witness

INFORMED CONSENT FORM - PART II

Clinical Investigation

Consent to Participate in Research Study

IRB # 813-90-8R4
PROTOCOL TITLE: Factors Affecting CNS Oxygen Toxicity in Humans.

The purpose of this study is to measure breathing and brain oxygen responses under conditions which might relate to the development of central nervous system oxygen toxicity. A certain proportion of people may develop toxic signs and symptoms when exposed to high oxygen concentrations under hyperbaric conditions. The classical signs and symptoms include: nausea, tunnel vision, ringing in the ears, tingling, numbness, irritability, and convulsions. The factors which determine the susceptibility of an individual to oxygen toxicity are not fully understood and are to be explored.

There will be a number of experimental phases which are listed and discussed below. You may participate in only one or in several of these phases. In the following listings, conditions which differ from a previous phase are shown in underlined and boldfaced type.

(a) - 0.11, 0.21, 1.75, and 2.8 ATM oxygen exposures
- 10 minute oxygen exposure durations
- carbon dioxide exposure progressing in 10 minutes from 0 to 8% sea level equivalent
- dry rest
- half day
- $75 compensation

(b) -**0.21, 1.75, and 2.8 ATM oxygen exposures**
- 10 minute oxygen exposure durations
- carbon dioxide exposure progressing in 10 minutes from 0 to 8% sea level equivalent
- **wet** rest
- half day
- $100 compensation

(c) - 0.21, 1.75, and 2.8 ATM oxygen exposures
- 10 minute oxygen exposure durations
- **carbon dioxide exposure for 10 min at 6.6% sea level equivalent**
- dry rest
- half day
- $100 compensation

(d) - 0.21, 1.75, and 2.8 ATM oxygen exposure
- **40 minute oxygen exposure duration at 1.75 ATM**
- **20 minute oxygen exposure duration at 2.8 ATM**
- dry **and wet** rest
- **AM and PM of two days**

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Initials

- $100 compensation

(e) - 0.21, 1.75, and 2.8 ATM oxygen exposure
- 40 minute oxygen exposure duration at 1.75 ATM
- **10 minute oxygen exposure with work at 2.8 ATM**
- dry and wet **work**
- AM and PM of two days
- $100 compensation

During the various phases of the study, you will be exposed to oxygen partial pressures including: 0.11, 0.21, 1.75, and 2.8 atmospheres (ATM). The normal oxygen partial pressure is 0.21 ATM or 21% of the atmospheric pressure at sea level. A partial pressure of 0.11 ATM is below normal and will make you breathe as though you are exercising heavily. You may also feel light-headed. To ensure that the oxygen content in your blood does not get too low, it will be monitored by a simple device which fits over your finger. Your response to low oxygen partial pressure will help to characterize your responses to high partial pressures. An oxygen partial pressure of 1.75 ATM is equivalent to breathing 100% oxygen at a depth of 25 feet of sea water (fsw) while a partial pressure of 2.8 ATM is equivalent to 100% oxygen at 60 fsw.

You will breathe from an apparatus which controls the oxygen and carbon dioxide partial pressures and measures the characteristics of your breathing. In Phases (a) and (b) listed above, the carbon dioxide partial pressure will rise to 7% sea level equivalent over a 10 minute period. During Phase (c), you will breathe carbon dioxide for 5 minutes after it rises to 5.8% sea level equivalent. Carbon dioxide will make you breathe as though you are exercising heavily. It also increases the risk of oxygen toxicity. While breathing gas containing carbon dioxide, 60% of our subjects have experienced symptoms which have included: sweating, narcosis, numbness, dizziness, feeling of imminent blackout, floating, irritability, tunnel vision, tingling, change in visual depth perception, ringing in the ears, headache, and anxiety. These symptoms have disappeared promptly upon breathing gas which does not contain carbon dioxide. If any such symptom (or unusual feeling) should occur at any time during any experiment, you should inform the Hyperbaric Center Tender who will accompany you in the chamber, and he will return you to breathing air.

The dry phases of the studies listed above will take place while you recline in a lawn-chair. During the phases (b), (d), and (e) which are wet, you will be immersed to the neck in warm water. While in the water, you will wear a safety helmet and a safety harness to prevent you from hitting your head or falling under water should you have a convulsion. Immersion increases the risk of oxygen toxicity.

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Initials

Phase (d) will investigate the duration of oxygen breathing while you are dry and immersed. You will breathe 1.75 ATM of oxygen for 40 minutes. The U.S. Navy allows 75 minutes of oxygen exposure at this partial pressure during heavy work in cold water. You will breathe 2.8 ATM of oxygen for 20 minutes. This is a common exposure during the Navy hyperbaric oxygen treatment tables.

Phase (e) will investigate exercise during wet and dry exposures. You will exercise on a bicycle ergometer either dry or immersed to the neck at a workload equivalent to a moderate jog. The exposure durations will be 40 minutes at 1.75 ATM oxygen partial pressure and 10 minutes at 2.8 ATM. The 2.8 ATM exposure is greater than the deepest exposure allowed by the U.S. Navy for operational diving which is 10 minutes at 2.52 ATM. You will be carefully monitored by the Tender during this time.

To avoid interactions between the various oxygen exposures during Phases (d) and (e), the 1.75 ATM exposure will be conducted in the morning and the 2.8 ATM exposure in the afternoon. There will be two experimental days with the first day dry and the second day wet.

The following paragraphs discuss the measurements that will be made. During Phases (a), (b), and (c), you will breathe from a mouthpiece connected to an apparatus which automatically measures your breathing characteristics. You will notice some resistance to heavy breathing.

During all experiments, you will wear a head-band which holds an infrared light transmitter and receiver tightly against your forehead. The pressure of the head-band may be slightly uncomfortable. Infrared light will be shined on your head, and a small amount will be reflected from your brain. The reflected light will allow us to assess the oxygenation of your blood and brain cells. The amount of infrared light you will be exposed to is approximately equal to the amount you experience on a sunny day. As far as we know, there are no ill effects from short exposures of this sort.

During the dry exposures of Phases (c) and (d), a short, flexible catheter will be placed in a vein of your arm and left there for the duration of the study (several hours). Placing the catheter will be slightly painful (as when blood is drawn), and there is a small of a bruise (bleeding under the skin). Accidental injection of the dye outside the vein (under the skin) due to incorrect needle placement can be painful. The risk of infection is small and severe infections are rare. Periodically (not more than six times) a small quantity of green dye (indocyanine green) will be injected into your vein, and its concentration will be measured in your brain by the infrared light probe on your head. A second probe may be placed on your neck to measure the dye concentration in an artery. The dye concentration information will be used to calculate blood flow to your brain. Green dye injections are performed routinely with minimal risk. The dye is

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Initials

eliminated within several minutes and has no toxic effect except that on rare occasions it may be associated with a possibly severe allergic response.

In summary, the major risk of this study is the potential for an oxygen convulsion. Convulsions can occur without warning and occasionally (but rarely) occur after a return from oxygen to air breathing. Oxygen convulsions are generally not harmful, and there have been no cases of neurological damage from uncomplicated convulsions despite many which have occurred. Impaired brain function or death are conceivable but are considered very unlikely from oxygen convulsions in a chamber. Complications can occur as a result of inhaling vomit or water, due to uncontrolled strenuous movement, and by striking hard objects. We will guard against these possibilities by having you wear a safety helmet and safety harness while in the water, by padding hard protrusions in the chamber, and by your avoidance of food and drink for two hours before a study. A convulsion lasts as long as several minutes during which you will stop breathing briefly and may bite your tongue. Your tongue also may interfere with your breathing. If you have a convulsion, the Tender who is with you will check your throat to ensure you are breathing properly. After a convulsion, you will be unconscious for a short period and may have a headache for several hours thereafter.

This study may have an adverse effect on an unborn child. Therefore, women of child-bearing potential must have a pregnancy test as indicated in Part I of this Consent Form.

I have read, understood, and have signed Part I of this Consent form. I have also read Part II and have been given the opportunity to discuss it and to ask questions. I agree to participate as a subject with the understanding that I may withdraw at any time.

Subject	Date	Witness	Date

APPENDIX B :

Dive Plan

This appendix contains the Dive Plan used for CNS oxygen toxicity experiments. It includes the procedure for handling the occurrence of a CNS oxygen toxicity seizure.

Dive Plan for Navy CNS O2 Toxicity Project

1. Inform Dr. Piantadosi or Dr. Moon that a Navy O2 toxicity experiment will be conducted.

2. Conduct dive in accordance with IRB-approved protocol. (See attachment.)

3. Decompress according to appropriate multi-level dive table for both the Tender and Subject. (See attachments.)

4. In the event of an oxygen seizure at 77 fsw:

Tender

(a) Remove Subject's mouthpiece and noseclip and have him breathe chamber air.

(b) Place padded tongue depressors crosswise in Subject's mouth, if possible, to prevent him from biting tongue.

(c) Restrain Subject to prevent injury on hard objects.

Chamber Operator

(a) Maintain chamber depth at 77 fsw.

(b) After the Subject is conscious and breathing normally and with the approval of the Diving Physician, decompress according to the appropriate air schedule. (See attachment.) Do not use oxygen decompression after a seizure.

APPENDIX C :

Decompression Tables

Multi-Level Dive Tables for Navy CNS O2 Toxicity Project

Oxygen Decompression (Estimated DCS Risk < 0.01%)

Level 1			Level 2			Decompression		
Depth	Time	Gas	Depth	Time	Gas	Depth	Time	Gas
77	20	Air	35	50	Air	30	5	O2
			35	60	Air	30	10	O2
77	30	Air	35	20	Air	30	5	O2
			35	30	Air	30	10	O2
			35	40	Air	30	10	O2
			35	50	Air	30	15	O2
			35	60	Air	30	20	O2
77	40	Air	-	-	-	10	5	O2
			-	-	-	30	5	O2
			35	20	Air	30	15	O2
			35	40	Air	30	25	O2
			35	60	Air	30	30	O2
77	60	Air	-	-	-	10	30	O2
			-	-	-	30	30	O2
			35	20	Air	30	35	O2
			35	40	Air	30	40	O2
			35	60	Air	30	45	O2
77	90	Air	-	-	-	10	60	O2
			-	-	-	30	60	O2

Compression: Not to exceed 75 fpm.

Decompression: 30 fpm to 30 fsw.
10 fpm from 30 fsw to surface.

Multi-Level Dive Tables for Navy CNS O2 Toxicity Project

Air Decompression (Estimated DCS Risk < 0.01%)

Level 1			Level 2			Decompression		
Depth	Time	Gas	Depth	Time	Gas	Depth	Time	Gas
77	20	Air	-	-	-		Note (a)	
			35	20	Air		Note (a)	
			35	30	Air		Note (a)	
			35	40	Air		Note (a)	
77	30	Air	-	-	-		Note (a)	
77	40	Air	-	-	-	10	65	Air
77	50	Air	-	-	-	10	180	Air
77	60	Air	-	-	-	10	250	Air
77	70	Air	-	-	-	10	300	Air
77	80	Air	-	-	-	10	340	Air
77	90	Air	-	-	-	10	370	Air

Compression: Not to exceed 75 fpm.

Decompression: 30 fpm to 30 fsw.
10 fpm from 30 fsw to surface.

Note (a): Breathe 100% oxygen from 30 fsw to surface unless an oxygen seizure has occurred.

APPENDIX D :

Incident Report - Oxygen Toxicity Seizure

September 9, 1992

Account of CNS Oxygen Toxicity Seizure in Subject MM

Protocol:

Eight experiments following the same protocol had been completed without incident, i.e., symptoms or seizure. The protocol is described below:

The subject is placed in a safety harness and hooked to a pulley attached to the ceiling of the chamber. Optical fibers residing in a headband are placed on the subjects head and a bicycle helmet is then strapped on over the headband. The subject climbs down a ladder onto a bicycle in the chamber below and assumes a comfortable position. The bicycle is used to attain a position consistent throughout all protocols of the study, including immersed exercise on the bicycle. The lights in the upper chamber are shut off to eliminate noise in the optical signals (lights remain on in the lower chamber). The optical signals measure cerebral oxygenation. The subject breathes from a mouthpiece connected to the spirometer-based closed-circuit ventilation system. The subject puts nose clips on and commences breathing. The subject is at rest for the entire study.

At the surface, the breathing medium is air equivalent, and is maintained at .2090 ATA oxygen. The CO_2 is allowed to rise by closing a valve which by-passes the CO_2 absorbent. When the end-tidal CO_2 reaches 50 torr (6.6 %), approximately 30 torr (4%) inspired, the CO_2 absorbent canister is opened partially in order to maintain P_{ETCO2} of 50 torr for a period of 10 minutes. Adjustments are made manually by the tender who also relays messages to the subject. After 10 minutes with a P_{ETCO2} = 50 torr, the by-pass is closed and full scrubbing is incorporated. The subject is informed of this and advised that breathing resistance may increase, typically from 4-5 cm H_2O to 8-10 cm H_2O. A two minute period follows during which the

P_{ETCO2} decreases to 30 - 40 torr end-tidal, and 0 inspired. At this point, the subject is told to come off the mouthpiece and the lights are turned on.

The chamber is then compressed to 77 fsw. The oxygen sensors (Teledyne micro fuel cells) and the CO_2 analyzer (Beckman LB2) are calibrated. The ventilation system is flushed with oxygen to bring the PIO_2 to 2.8 ATA (85% O_2). The procedure is then repeated. After completing this portion of the study, the chamber is decompressed to 35 fsw and the procedure is repeated with a constant PIO_2 of 1.75 ATA (85% O_2).

After measurements are finished at 35 fsw the subject climbs out of the lower chamber and decompression to 30 fsw is initiated. Both the subject and tender breathe oxygen according to the Multilevel Decompression Tables designated in the IRB approved protocol.

Study on 9/9/92 - Subject MM

The surface run was consistent with the previous eight studies with the following exceptions:

1) The period of CO_2 accumulation lasted approximately 7 minutes whereas the average time for previous subjects was approximately 4-5 minutes. The CO_2 analyzer calibration was checked at 6 minutes and proved to be valid.
2) The spirometer filled over capacity and volume was removed. This normally occurs at higher PIO_2's in the initial phase of the experiment at depth in order to attain the high oxygen set point. The reason for increased volume was thought to be an inward leak through the subject's nose. He reported having to adjust them frequently during the surface run.
3) The optical signals corresponded to increases in cerebral oxygenation greater than in previous studies under the same conditions.
4) This subject's ventilatory response to CO_2 was lower than that of subjects in previous studies under the same conditions.
5) After the surface study, the subject reported headache, anxiety, sweating, and labored breathing. Each of these sensations have been reported by past subjects under the same conditions and are consistent with breathing gas with elevated CO_2.

Compression to 77 fsw took longer than in previous studies (11.5 minutes vs 6 minutes) because the subject had problems clearing his ears. He had used Affrin prior to the surface run because he has had trouble equalizing in the past.

The run at 77 fsw was consistent with the previous 8 studies done under the same conditions until the seizure with the following comments:

1) Calibration of the sensors took approximately 12 minutes which is consistent with previous studies.
2) The tender and chamber operator were informed of the large optical signal changes and low ventilation in the surface run which in my opinion could predispose a person to CNS oxygen toxicity.
3) After the subject went on the mouthpiece, 2 minutes were required to raise the PIO_2 to 2.8 ATA.
4) Approximately 5 minutes were required to build up the end-tidal CO_2 to 50 torr at which point the 10 minutes of constant CO_2 was started.
5) Again the optical signal changes were large and ventilatory response to CO_2 appeared to be small compared to earlier subjects.
6) The ten minutes ended normally. The P_{ETCO2} range was 42 - 52, typically 50 torr with short excursions to the range limits. The subject was informed that the CO_2 scrubbing was initiated and may cause an increase in breathing resistance (approx. 10 cm H_2O peak to peak). The chamber operator was notified that we would be ready to decompress in 2 - 3 minutes.
7) The seizure occurred 1 minute after full scrubbing was employed. The PIO_2 was 2.83 ATA and $ETCO_2$ was 35 torr (inspired CO_2 was approximately 0). The chamber had been at 77 fsw for approx. 40 minutes. The subject had been on gas with a PIO_2 of 2.8 for approx. 18 minutes.

Seizure:

The subject yelled and began shaking vigorously. The tender communicated that the subject was seizing. From the porthole in Delta, his head was barely visible but the shaking of the safety harness was evident. The lights were turned on. The tender was on the headset but after being unable to pull the subject up via the pulley system the headset fell off as he went into the lower chamber. The chamber operator asked about the medical officer and was told to get the physician. The outside tender switched to overhead communications and told the inside tender to get the subjects mouthpiece out of his mouth. The mouthpiece had fallen out of the subject's mouth immediately upon onset of the seizure but this fact was unclear at the time.

Topside personnel were informed that the inside tender needed assistance and a PA, an RN, and a physician were blown down to Foxtrot, duration was approximately 3 min. Approximately 4 - 5 minutes had

transpired between seizure onset and arrival of help to Delta. An ambu-bag and 2 O_2 masks were locked into Foxtrot. A stethoscope was locked down to Delta. When the chambers were mated and the subject was no longer shaking but was unresponsive and was laid on the floor in Delta chamber and confirmed to be breathing normally. Eyes were moving with left to right nystagmus at about 1 second rate. The subject was still salivating. Breathing was regular at a rate of about 20 - 30, heart rate was around 120. Lungs were clear bilaterally. After approximately 5 minutes in this condition, the patient did respond by opening eyes wider on command. He did not squeeze hands or move extremities to command. Approximately 12 minutes after seizure onset, the subject would minimally follow commands such as moving right and left arms. He was unable to communicate verbally and any attempt at speech was very dysarthric and difficult to understand. Approximately 15 minutes post-seizure, the patient was verbally responding although he was difficult to understand. Breathing was regular, heart rate was in the 90's, lungs were clear and no obvious focal neurological deficits were observed. At that time the principal investigator was informed of the incident and decompression was initiated. Upon reaching the surface a more thorough neurological exam was performed and was found to be normal. The patient complained of fatigue, a slight headache, and was slightly confused in that he could not remember where his class was that afternoon. He was oriented to person, place and time. After approximately one hour, the subject had no complaints other than fatigue. He was able to remember his class. He was observed for another hour and driven home where several follow calls were made that night without incident.

APPENDIX E :

Ventilatory and Circulatory Data

Phase 1
Ventilatory Response Slopes

VENTILATORY RESPONSE SLOPES (l/min/torr)			
SUBJECT	0.21 (ata) PIO2	1.75 (ata) PIO2	2.80 (ata) PIO2
DV	2.49	6.07	2.19
TA	1.78	0.96	0.79
DV	2.15	4.00	3.66
TA	1.23	2.07	1.12
CM	2.58	1.53	0.91
DV	2.49	4.47	3.73
CM	2.49	2.70	1.30
CD	2.86	2.78	1.98
CM	4.48	3.20	2.25
CD	2.20	2.43	1.32
CP	2.98	2.93	1.81
DS	2.25	2.09	2.08
DS	1.72	3.24	3.38
CD	2.00	3.13	1.51
CM	3.19	2.88	2.24
CD	1.37	2.21	1.60
CM	1.91	2.90	2.17
TA	1.52	1.83	1.21
AE	0.93	2.38	0.66
CM	2.07	2.42	2.23
AE	1.64	2.85	1.71
SH	1.14	0.85	0.80
AE	1.07	2.95	1.53
CM	2.86	3.10	2.81
CM	2.50	1.96	1.63
CD	0.68	1.16	1.34
DS	2.61	2.54	2.96
AE	1.25	1.97	1.20
CP	2.69	2.86	1.49
TS	2.13	2.14	2.60
TOTAL			
AVG	2.11	2.62	1.87
SD	0.80	1.03	0.83
W HYPOXIA			
AVG	2.30	2.73	1.91
SD	0.89	1.33	0.93
W/O HYPOXIA			
AVG	1.90	2.54	1.79
SD	0.70	0.66	0.73

Phase 1
Ventilatory Response Duration (min)

Subject	Duration (min)			
	0.11 (ata)* PIO2	0.21 (ata) PIO2	1.75 (ata) PIO2	2.80 (ata) PIO2
DV	10.48	6.5	9	5.25
TA	12.17	6	10	7
DV	12.43	5.1	11	6
MN	7.52	4	8.5	6
TA	6.01	5	13.5	5
DV	9.93	4	12.5	7
CM	7.02	4	7.05	3
OD	7.3	4	9	5
MN	9.9	7	9	9.5
CM	8.6	4.5	8	5
DS	12.1	4	10.5	5
DS	8.1	3.5	11.5	4.5
CM	10.31	5	10	4
OD	8.43	4.5	5	6.5
CM	8.86	4.5	11	5.5
TA	7.4	4.5	7	5
CM		4.5	5	4
AE		3.5	6	3.5
AE		4.5	8	5.5
CM		4.5	8	5.5
CM		4	8	4
OD		4.5	8	5
DS		5.5	8.5	5
TS		9	14	5
AE		4	6	4.5
CP		4.5	9	7
AE		4	6	4
SH		6	6.5	6
OD		4.5	7	6
CP		4	9.5	6
OD		5	9	5.5
CM		4.5	5.5	5
AVG	9.16	4.77	8.64	5.34
SD	1.97	1.12	2.32	1.24

* Average PIO2 over ventilatory response duration
PIO2 range 0.21 to 0.05 ata

Phase 2
Ventilatory Response Slopes

Ventilatory Response Slope (liter/min/torr)			
SUBJECT	0.21 (ata) PIO2	1.75 (ata) PIO2	2.80 (ata) PIO2
DD	0.74	1.21	0.36
JN	1.12	0.59	0.65
JR	4.84	2.81	2.15
MR	1.57	1.10	1.69
OD2	4.94	1.27	1.18
OD3	3.22	1.58	1.49
TS	4.65	1.77	1.00
WW	0.85	0.64	0.51
BW	2.30	1.27	0.90
TA	2.97	1.41	1.07
CM	3.94	2.33	2.39
DV	3.92	2.90	2.16
OD4	3.43	1.71	1.61
CM2	4.23	2.70	1.99
DD2	0.96	0.98	1.02
DV2	2.98	2.46	1.82
JN2	1.48	1.01	1.24
TA2	2.19	3.03	1.19
MR2	1.69	0.96	0.50
PF	1.76	0.83	0.82
PF2	1.54	0.98	0.96
BW2	1.42	1.10	0.60
WW2	0.73	0.47	0.34
PF3	2.46	1.72	1.03
OD5	3.30	1.39	1.29
BC1	4.31	4.06	1.39
PF4	2.62	1.63	0.97
BC2	4.69	2.08	1.37
WET			
AVG	2.66	1.60	1.22
SD	1.36	0.93	0.58
DRY			
AVG	2.35	1.58	1.12
SD	1.27	0.83	0.52
SYMPTOMS			
AVG	2.45	1.65	1.17
SD	0.75	0.64	0.29
NO SYMPTOMS			
AVG	2.80	1.64	1.22
SD	1.64	0.99	0.67
TOTAL			
AVG	2.67	1.64	1.20
SD	1.38	0.87	0.56

Phase 2
Ventilatory Response Duration (min)

Subject	Duration (min)		
	0.21 (ata) PIO2	1.75 (ata) PIO2	2.8 (ata) PIO2
OD2	7.78	7.60	6.98
DD	7.37	11.19	7.95
JN	5.10	7.95	7.43
JR	8.50	10.03	8.53
TS	7.84	9.60	6.55
WW	4.95	7.18	6.33
MR	6.03	7.05	6.48
OD3	6.45	8.28	7.78
CM	6.33	6.21	7.05
TA	6.47	6.15	3.22
DV	6.33	6.02	6.26
BW	8.22	6.83	7.72
OD4	6.25	6.50	6.50
CM2	4.28	5.28	5.92
DD2	3.62	5.80	5.11
DV2	5.62	5.67	6.02
JN2	5.72	5.97	7.15
TA2	5.97	8.10	6.17
MR2	4.50	6.54	5.84
WW2	4.43	4.84	4.82
PF	6.97	6.80	7.60
PF2	9.63	9.25	8.70
BW2	5.50	6.10	6.37
PF3	7.25	6.49	6.20
OD5	5.58	7.42	7.72
BC	8.28	11.08	9.29
PF4	8.13	8.96	8.50
BC2	8.67	9.83	8.91
TOTAL			
AVG	6.49	7.45	6.90
SD	1.52	1.74	1.34
WET			
AVG	7.19	8.14	7.23
SD	1.36	1.61	1.42
DRY			
AVG	5.69	6.66	6.52
SD	1.31	1.58	1.19
SYMPTOMS			
AVG	6.69	7.30	6.71
SD	1.69	1.30	1.70
NOSYMPTOMS			
AVG	6.36	7.57	6.95
SD	1.41	1.92	1.12

Phase 3
Ventilatory Response Slopes

Ventilatory Response Slope (liter/min/torr)			
SUBJECT	0.21 (ata) PIO2	1.75 (ata) PIO2	2.80 (ata) PIO2
AW	2.50	0.79	0.86
BB	1.87	0.54	1.12
CM	3.27	1.53	2.09
DD	1.65	0.34	0.54
FC1	2.00	1.70	0.46
FC2	1.82	1.31	0.80
KS	0.82	0.39	0.36
PF	2.08	1.57	1.68
MR	2.94	1.80	0.90
MM	0.60		0.25
AVG	1.95	1.11	0.91
SD	0.83	0.59	0.59

Phase 3
Ventilatory Parameters

Subject	PETCO2 (torr)			Ventilation (liter/min) BTPS		
	0.21 (ata) PIO2	1.75 (ata) PIO2	2.80 (ata) PIO2	0.21 (ata) PIO2	1.75 (ata) PIO2	2.80 (ata) PIO2
	Control			Control		
AW	39.25	30.20	38.40	21.61	9.53	24.27
BB	34.48	34.07	35.41	16.29	19.94	20.89
CM	41.83	37.10	41.95	14.96	22.75	14.66
DD	45.25	36.20	25.03	27.17	16.76	21.18
FC1	33.30	34.15	42.63	12.08	22.42	18.80
FC2	38.03	35.96	31.09	12.62	17.52	16.90
KS	40.10	34.88	36.88	7.58	18.34	17.52
MM	34.73		32.74	15.58		15.08
MR	35.70	38.97	37.37	17.52	21.06	21.81
PF	36.62	34.80	33.50	18.14	23.13	18.28
	Start PETCO2=50 torr			Start PETCO2=50 torr		
AW	49.55	51.75	47.30	50.93	29.53	35.00
BB	50.33	51.08	51.18	35.21	27.61	29.70
CM	48.50	50.28	51.78	49.88	43.48	26.98
DD	49.90	49.63	49.20	44.65	22.64	26.95
FC1	46.78	49.30	51.33	32.55	32.05	28.72
FC2	50.23	50.33	49.58	34.77	28.58	30.08
KS	50.75	50.63	51.78	16.40	15.44	13.40
MM	49.88		48.23	31.71		21.43
MR	49.60	50.20	47.38	55.18	45.74	34.62
PF	50.73	50.47	49.23	38.42	35.73	43.04
	End PETCO2=50 torr			End PETCO2=50 torr		
AW	49.43	50.15	51.43	63.07	30.09	24.86
BB	49.10	50.30	51.03	54.89	30.03	29.92
CM	49.78	50.28	50.98	69.40	54.55	33.06
DD	49.93	50.15	49.78	40.54	22.97	23.94
FC1	50.05	50.68	50.65	43.77	36.23	27.15
FC2	50.05	50.90	51.45	34.36	29.14	29.78
KS	50.10	48.83	51.33	19.22	20.88	15.07
MM	47.85		48.38	36.12		22.16
MR	45.83	47.55	48.33	61.45	44.02	46.61
PF	50.10	50.58	52.40	52.01	40.51	40.31

Phase 4
Ventilatory Parameters

Subject	PETCO2 (torr)				Ventilation (liter/min) BTPS			
	0.21 (ata) PIO2	1.75 (ata) PIO2	0.21 (ata) PIO2	2.80 (ata) PIO2	0.21 (ata) PIO2	1.75 (ata) PIO2	0.21 (ata) PIO2	2.80 (ata) PIO2
	Start				**Start**			
AW	33.19	32.01	34.72	27.99	13.22	12.80	13.45	11.34
FC	32.33	31.52	34.35	26.57	13.73	13.44	11.81	15.21
PF	33.86	25.84	30.00	26.78	11.93	9.97	9.85	10.83
PS	32.73	31.13	33.24	29.09	13.69	9.59	14.39	11.15
BFC	29.46	29.49	29.83	26.15	13.07	18.91	12.01	17.96
BC	29.11	32.15	31.29	29.87	15.09	15.35	12.89	12.32
CI	36.08	32.39	38.99	31.14	15.14	15.94	14.76	17.26
CM	36.85	28.04	37.10	28.66	13.14	14.58	16.62	14.83
KS	38.81	31.41	37.11	34.86	8.99	10.07	9.87	8.91
RT	36.01	32.09	34.78	29.83	12.39	16.09	13.40	12.05
CW	35.48	27.64	29.80	24.43	10.68	16.84	13.24	17.71
	End				**End**			
AW	NA	30.18	NA	28.70	NA	13.13	NA	10.67
FC	NA	31.35	NA	26.30	NA	15.92	NA	12.20
PF	NA	24.98	NA	27.33	NA	14.28	NA	10.94
PS	NA	33.70	NA	27.40	NA	12.61	NA	11.52
BFC	NA	29.00	NA	25.70	NA	10.98	NA	12.64
BC	NA	33.33	NA	30.88	NA	9.93	NA	12.42
CI	NA	29.43	NA	30.98	NA	12.26	NA	14.29
CM	NA	27.55	NA	27.35	NA	18.13	NA	15.13
KS	NA	29.78	NA	33.65	NA	11.26	NA	10.44
RT	NA	30.73	NA	29.20	NA	14.09	NA	12.36
CW	NA	27.80	NA	24.03	NA	16.40	NA	14.34

Phases 5 and 6
Ventilatory Parameters

Subject	PETCO2 (torr)						Ventilation (lpm) BTPS					
	0.21 (ata) PIO2	1.75 (ata) PIO2		0.21 (ata) PIO2	2.80 (ata) PIO2		0.21 (ata) PIO2	1.75 (ata) PIO2		0.21 (ata) PIO2	2.80 (ata) PIO2	
	Control	Start	End	Control	Start	End	Control	Start	End	Control	Start	End
Phase 5 - Head-out Immersion							**Phase 5 - Head-out Immersion**					
AW	31.82	33.60	29.98	31.76	32.33	31.48	15.52	11.81	11.39	14.32	10.17	10.48
CM	37.54	28.83	28.76	36.54	31.65	27.38	18.85	12.77	12.79	12.08	16.65	14.43
CW	33.17	25.10	25.90	33.98	25.50	23.05	13.63	13.43	11.99	11.73	15.97	16.25
FC	37.76	30.78	35.93	36.64	34.58	35.28	11.86	9.58	14.92	10.90	11.10	12.98
KS	39.56	34.25	32.33	39.25	31.93	30.55	8.55	9.01	8.74	9.45	11.97	9.43
RT	36.66	35.98	31.74	37.79	27.03	24.58	10.44	11.09	12.23	13.87	18.88	11.52
Phase 6 - Total Immersion							**Phase 6 - Total Immersion**					
CI	31.82	33.78	33.73	31.76	33.40	31.28	15.52	14.70	12.38	14.32	15.10	15.23
PF	37.54	33.45	28.13	36.54	30.08	27.23	18.85	10.04	10.87	12.08	12.97	17.98
HM	33.17	36.13	36.28	33.98	33.13	31.62	13.63	11.50	12.00	11.73	10.88	11.85
FC	37.76	35.83	27.18	36.64	33.50	30.73	11.86	15.21	11.36	10.90	11.90	10.67
RS	39.56	33.75	28.16	39.25	28.85	24.90	8.55	13.85	13.39	9.45	8.43	6.81
MR	36.66	33.33	32.73	37.79	35.28	28.93	10.44	12.52	12.82	13.87	17.07	14.93

Phase7
Ventilatory Parameters
and Heart Rate

Subject	PETCO2 (torr)						Ventilation (lpm) BTPS						Heart Rate (bpm)					
	0.21 (ata) PIO2			2.80 (ata) PIO2			0.21 (ata) PIO2			2.80 (ata) PIO2			0.21 (ata) PIO2			2.80 (ata) PIO2		
	HO	Total	Rec	HO	Total	Rec	HO	Total	Rec	HO	Total	Rec	HO	Total	Rec	HO	Total	Rec
BL	29.73	31.29	30.50	26.33	26.60	23.83	12.50	10.73	13.10	11.15	10.72	14.89	67	79	77	62	72	67
DL	25.88	27.97	29.29	30.90	32.04	33.23	21.62	13.93	13.29	12.38	10.46	10.10	90	87	83	70	72	70
FC	32.65	34.35	26.20	30.37	31.96	28.90	13.97	10.00	9.44	14.22	11.42	11.36	63	61	58	52	48	50
HM	34.77	35.91		28.75	29.98		10.85	10.95		12.44	11.63							
JB	34.03	32.97		30.34	31.77		12.31	13.66		13.85	11.68							
JH	35.21	32.00	34.05	31.11	27.04	27.15	13.22	14.58	12.73	13.11	14.24	14.32	75	79	80	66	71	67
MR	37.04	36.32	36.70	32.76	33.10	32.77	12.97	12.96	14.71	14.02	13.38	13.16						
RF	29.74	30.91	30.79	32.80	31.68	31.66	20.63	12.74	14.13	14.79	10.50	10.04	91	90	92	91	90	92
RS	33.85	32.71	32.98	28.17	27.46	25.92	20.92	13.76	15.68	15.86	14.07	14.96	91	89	88	79	76	72
RT	39.17	36.24		30.27	28.50		13.71	12.88		15.23	14.68							

Phase 8
Ventilatory Parameters
and Heart Rate

Subject	PETCO2 (torr)						Heart Rate (bpm)					
	0.21 (ata) PIO2						0.21 (ata) PIO2					
	HO	BH/HO	IM	REC	IM	IM/BH	HO	BH/HO	IM	REC	IM	IM/BH
TA	35.92	NA	33.96	30.62	26.09	NA	61	55	62	59	66	60
RS	29.50	NA	36.51	35.71	36.13	NA	83	64	92	81	90	69
FC	35.51	NA	35.50	32.93	31.73	NA	67	58	75	73	70	64
JB	26.94	NA	23.84	24.49	17.91	NA	81	70	70	61	83	65
HM	35.46	NA	35.17	34.48	34.51	NA	80	65	72	75	84	60
CI	37.68	NA	39.95	39.11	39.67	NA	60	56	82	63	75	87

Phases 9 and 10
Ventilatory Parameters

Sub.	Oxygen Consumption (lpm)						PETCO2 (torr)						Ventilation (lpm) BTPS						Heart Rate (bpm)			
	0.21 (ata) PIO2			1.75 (ata) PIO2			0.21 (ata) PIO2			1.75 (ata) PIO2			0.21 (ata) PIO2			1.75 (ata) PIO2			0.21 (ata) PIO2		1.75 (ata) PIO2	
	Rest	Start	End	Rest	Start	End	Rest	Start	End	Rest	Start	End	Rest	Start	End	Rest	Start	End	Rest	Work	Rest	Work
Phase 9 - Dry - Work																						
BP	1.30	1.81	1.79	1.64	2.43	2.46	32.4	41.9	39.2	30.1	39.8	41.5	14.9	33.4	43.3	23.9	36.5	35.8	87	114		
CI	1.42	1.07	1.32	1.37	1.21	1.47	35.2	37.8	38.1	31.1	36.1	42.1	13.3	17.8	18.4	17.8	25.3	38.2				
FC	1.27	1.61	1.67	1.17	1.69	1.86	36.4	41.9	43.4	37.5	47.0	49.6	15.5	29.1	33.7	21.7	32.4	34.7				
KB	2.09	3.58	3.61	1.81	1.92	2.40	32.5	36.4	38.5	30.7	34.5	38.6	17.6	32.9	40.2	20.8	34.0	44.9	100	114	79	109
KS	0.76	1.14	1.21	1.44	0.89	0.86	38.4	38.6	39.2	33.6	42.0	47.7	7.7	11.6	12.2	12.9	17.7	23.2	91	118	102	112
PD	1.25	1.57	1.76	1.48	1.46	1.73	37.0	38.9	39.6	32.9	38.4	37.0	15.2	20.2	24.3	17.3	29.5	32.3				
PF	1.19	1.88	1.79	0.55	1.51	2.02	35.1	40.9	41.8	31.9	40.7	47.4	10.2	26.4	33.0	11.8	28.8	38.2	75	97	68	97
RS	0.76	1.53	1.74	0.98	1.48	1.85	31.9	35.6	36.2	34.4	37.7	41.9	13.8	13.7	13.8	12.8	16.8	22.5				
RT	2.37	1.98	2.22	2.15	3.26	3.08	39.0	43.7	44.1	35.5	41.6	47.3	12.0	40.6	38.7	11.5	29.0	36.9	80	110	65	103
Phase 10 - Total Immersion - Work																						
CI	1.14	1.79	1.89	1.05	1.56	1.72	35.0	38.9	39.0	35.7	37.3	38.5	17.9	17.3	17.3	18.3	20.8	23.7	79	90	66	77
FC	1.76	1.80	2.19	1.17	2.29	2.32	33.5	38.6	38.2	24.4	37.3	38.1	17.3	18.0	16.9	10.0	17.7	17.5	70	81	63	72
JB	1.22	1.85	2.37	1.39	2.10	2.16	36.8	36.8	36.4	26.4	35.0	38.9	19.9	19.7	20.9	17.9	24.4	27.7	71	77	59	69
HM	0.69	1.52	1.76	1.04	1.92	1.78	35.3	34.9	35.2	30.0	29.2	31.0	11.1	13.0	15.5	12.7	16.0	17.9	86	89	77	84
PD	1.21	1.84	1.59	1.06	1.46	1.17	31.6	33.4	36.3	32.8	35.2	39.2	14.3	15.2	16.8	12.5	19.6	24.1	52	82	48	77
PF	0.89	1.42	1.15	1.06	1.43	1.38	26.7	29.2	35.8	28.5	36.6	35.8	18.4	15.1	25.5	15.6	28.4	29.8	73	94	66	93
RF	1.01	1.41	1.12	0.64	1.40	1.68	33.0	36.6	37.5	34.0	41.5	44.4	16.0	16.9	16.5	14.0	22.5	27.0	90	101	78	99
RS	1.36	1.51	1.37	1.52	1.83	1.58	36.6	34.1	39.0	25.9	25.1	25.7	13.8	16.5	19.4	17.2	23.6	19.8	92	95	80	95
RT	1.17	1.59	1.09	1.26	1.45	1.61	34.3	38.1	39.0	37.6	37.8	38.3	20.2	22.5	22.5	18.4	28.9	37.9	76	89	64	84

APPENDIX F :

Cerebral Oxygenation Data

Phase 1

Deoxygenated Hemoglobin (tHb)

SUBJECT	Change Relative to Control (v.d.)						
	0.05 (ata) PIO2	0.21 (ata) PIO2	0.21 (ata) PIO2	1.75 (ata) PIO2	1.75 (ata) PIO2	2.80 (ata) PIO2	2.80 (ata) PIO2
			60 (torr) PETCO2		60 (torr) PETCO2		60 (torr) PETCO2
DV	0.2759	0	-0.0702	-0.2225	-0.283	-0.2116	-0.3086
TA	0.1748	0	-0.0617	-0.1893	-0.2547	-0.1601	-0.2688
DV	0.2376	0	-0.0377	-0.1188	-0.2626	-0.1731	-0.3076
MN	0.4198	0	-0.0578	-0.1708	-0.307	-0.2418	-0.3364
TA	0.2698	0	-0.0522	-0.3367	-0.4976	-0.3611	-0.4679
CM	0.2716	0	-0.0767	-0.3141	-0.5042	-0.4808	-0.5062
DV	0.3774	0	-0.0287	-0.2965	-0.3509	-0.2757	-0.3186
CM	0.2333	0	-0.0973	-0.1148	-0.2233	-0.1347	-0.1858
CD	0.1992	0	-0.0092	-0.1232	-0.1852	-0.1123	-0.1828
MN	0.1507	0	-0.0209	-0.0932	-0.184	-0.0507	-0.1283
CM	0.1786	0	-0.0732	-0.2567	-0.3048	-0.2335	-0.256
CD	0.2959	0	-0.0505	-0.0648	-0.1419	-0.1439	-0.1894
CP	0.3465	0	-0.1051	-0.1694	-0.2508	-0.1483	-0.23
DS	N/A	0	-0.0003	-0.1224	-0.2251	-0.1977	-0.2452
DS	N/A	0	-0.0202	-0.1683	-0.3107	-0.2007	-0.313
CD	N/A	0	-0.0487	-0.0155	-0.0597	-0.2479	-0.1363
CM	N/A	0	-0.0809	-0.2043	-0.3153	-0.2282	-0.2656
CD	N/A	0	-0.0084	-0.5554	-0.497	-0.3237	-0.4787
CM	N/A	0	-0.1159	-0.0738	-0.3316	-0.1122	-0.2206
TA	N/A	0	-0.0875	-0.1371	-0.3373	-0.0971	-0.23
AE	N/A	0	-0.0706	-0.2205	-0.302	-0.2026	-0.301
CM	N/A	0	-0.0433	-0.1174	-0.242	-0.1824	-0.2603
AE	N/A	0	-0.06	-0.2147	-0.3479	-0.3076	-0.3905
SH	N/A	0	-0.1088	-0.2616	-0.1629	-0.1239	-0.1524
AE	N/A	0	-0.0705	-0.1603	-0.3614	-0.1993	-0.3928
CM	N/A	0	-0.0302	-0.1307	-0.3508	-0.1415	-0.2148
TA	N/A	0	-0.0314	-0.1107	-0.1107	-0.1832	-0.2527
CM	N/A	0	-0.0436	-0.1481	-0.2574	-0.2226	-0.24762
CD	0.1579	0	-0.0503	-0.1045	-0.1173	-0.2003	-0.3114
DS	0.288	0	-0.0265	-0.0126	-0.1777	-0.0664	-0.183
TS	0.193	0	-0.0477	0.0092	-0.071	-0.0958	-0.1946
AE	-0.0423	0	-0.0191	-0.0578	-0.0578	-0.5453	-0.5936
CP	N/A	0	-0.0106	0.0741	-0.0021	-0.0487	-0.1027

Phase 1
Oxygenated Hemoglobin (tHbO2)

SUBJECT	Change Relative to Control (v.d.)						
	0.05 (ata) PIO2	0.21 (ata) PIO2	0.21 (ata) PIO2 60 (torr) PETCO2	1.75 (ata) PIO2	1.75 (ata) PIO2 60 (torr) PETCO2	2.80 (ata) PIO2	2.80 (ata) PIO2 60 (torr) PETCO2
DV	-0.2261	0	0.1184	0.1807	0.2918	0.1997	0.3473
TA	-0.1516	0	0.109	0.065	0.1681	0.0434	0.2534
DV	-0.156	0	0.0596	-0.0573	0.1221	0.1133	0.2765
MN	-0.4437	0	0.1494	0.0232	0.2961	0.1427	0.3345
TA	-0.3211	0	0.1418	0.2319	0.5591	0.2642	0.6915
CM	-0.1971	0	0.2915	0.2252	0.5009	0.4273	0.5651
DV	-0.2758	0	0.0647	0.1254	0.2569	0.1864	0.4022
CM	-0.1866	0	0.1788	0.0494	0.2865	0.0627	0.1958
CD	-0.2034	0	0.0215	0.0934	0.1925	0.0897	0.1763
MN	-0.0777	0	0.1343	0.0697	0.221	-0.0203	0.0992
CM	-0.1725	0	0.2266	0.1643	0.5315	0.1646	0.4557
CD	-0.2684	0	0.0972	-0.1493	0.0097	-0.0886	0.0504
CP	-0.4269	0	0.206	-0.3317	-0.2402	-0.319	-0.1551
DS	N/A	0	0.045	-0.02	0.1294	0.1685	0.2849
DS	N/A	0	0.04	0.1377	0.306	0.1693	0.2756
CD	N/A	0	0.1115	0.115	0.2366	0.1915	0.2182
CM	N/A	0	0.1658	0.0707	0.5019	0.1071	0.2585
CD	N/A	0	0.1111	0.4506	0.4659	0.2787	0.4373
CM	N/A	0	0.2433	-0.0668	0.5108	-0.1434	0.1569
TA	N/A	0	0.1331	0.2124	0.4252	0.1089	0.2527
AE	N/A	0	0.1558	0.1329	0.3012	0.0611	0.24
CM	N/A	0	0.0798	0.0396	0.3554	0.0081	0.2683
AE	N/A	0	0.1165	0.1255	0.3264	0.2646	0.4355
SH	N/A	0	0.247	0.4823	0.2128	0.0492	0.1693
AE	N/A	0	0.1507	0.0577	0.4603	0.1298	0.5016
CM	N/A	0	0.0304	0.28	0.6526	0.0866	0.2463
TA	N/A	0	0.052	-0.0545	-0.0545	-0.015	0.111
CM	N/A	0	0.1318	0.0505	0.3531	0.1108	0.2919
CD	-0.0984	0	0.2223	0.1448	0.211	0.3491	0.4263
DS	-0.1441	0	0.0393	-0.1503	0.0293	-0.0818	0.0876
TS	-0.1359	0	0.1036	-0.0641	0.0654	0.0633	0.262
AE	0.2167	0	0.0384	0.2593	0.2593	0.6096	0.6831
CP	N/A	0	0.0819	-0.0733	0.019	0.0902	0.2002

Phase 1
Blood Volume (tBV)

SUBJECT	Change Relative to Control (v.d.)						
	0.05 (ata) PIO2	0.21 (ata) PIO2	0.21 (ata) PIO2 60 (torr) PETCO2	1.75 (ata) PIO2	1.75 (ata) PIO2 60 (torr) PETCO2	2.80 (ata) PIO2	2.80 (ata) PIO2 60 (torr) PETCO2
DV	0.0496	0	0.048	-0.0419	0.0087	-0.012	0.0385
TA	0.0232	0	0.0471	-0.1243	-0.0866	-0.1167	-0.0194
DV	0.0815	0	0.0219	-0.176	-0.1404	-0.0597	-0.031
MN	-0.024	0	0.0917	-0.1477	-0.011	-0.0991	-0.0019
TA	-0.0148	0	0.0896	-0.1047	0.0615	-0.0969	0.2235
CM	0.0745	0	0.2148	-0.0889	-0.0033	-0.0527	0.0589
DV	0.1016	0	0.0361	-0.171	-0.0938	-0.0892	0.0837
CM	0.0467	0	0.0816	-0.0654	0.0632	-0.072	0.0099
CD	-0.0042	0	0.0122	-0.0297	0.0073	-0.0226	-0.0066
MN	0.073	0	0.1134	-0.0235	0.0362	-0.071	-0.0291
CM	0.0069	0	0.1542	-0.0915	0.2275	-0.068	0.2005
CD	0.0275	0	0.0467	-0.214	-0.1322	-0.2325	-0.1389
CP	-0.0805	0	0.1009	-0.501	-0.4909	-0.4672	-0.385
DS	N/A	0	0.0446	-0.1423	-0.0957	-0.0292	0.0397
DS	N/A	0	0.0199	-0.0305	-0.0047	-0.0314	-0.0374
CD	N/A	0	0.0627	0.0994	0.1767	-0.0564	0.0819
CM	N/A	0	0.0848	-0.1336	0.1865	-0.1211	-0.007
CD	N/A	0	0.1026	-0.1049	-0.0311	-0.0451	-0.0415
CM	N/A	0	0.1273	-0.1406	0.179	-0.2556	-0.0637
TA	N/A	0	0.0455	0.0752	0.0879	0.0117	0.0226
AE	N/A	0	0.0851	-0.0876	-0.0009	-0.1415	-0.061
CM	N/A	0	0.0365	-0.0778	0.1135	-0.1743	0.008
AE	N/A	0	0.0566	-0.0892	-0.0214	-0.0429	0.045
SH	N/A	0	0.1382	0.2207	0.0498	-0.0746	0.0168
AE	N/A	0	0.0803	-0.1027	0.0989	-0.0695	0.1087
CM	N/A	0	0.0003	0.1493	0.3018	-0.0549	0.0314
TA	N/A	0	0.0205	-0.1653	-0.1653	-0.1983	-0.1419
CM	N/A	0	0.0883	-0.0975	0.0957	-0.1117	0.0449
CD	-0.0667	0	0.0456	-0.0859	-0.0327	0.0224	-0.0115
DS	0.1438	0	0.0129	-0.163	-0.1484	-0.1481	-0.0954
TS	0.057	0	0.0559	0.0352	-0.0055	-0.0324	0.0674
AE	0.1745	0	0.0193	0.2015	0.2015	0.0643	0.0896
CP	N/A	0	0.0713	0.0007	0.0168	0.0415	0.0975

Phase 1

Cytochrome a,a 3 (CYT)

SUBJECT	Change Relative to Control (v.d.)						
	0.05 (ata) PIO2	0.21 (ata) PIO2	0.21 (ata) PIO2 60 (torr) PETCO2	1.75 (ata) PIO2	1.75 (ata) PIO2 60 (torr) PETCO2	2.80 (ata) PIO2	2.80 (ata) PIO2 60 (torr) PETCO2
DV	-0.0909	0	-0.0047	0.0331	0.0197	0.0745	0.1029
TA	-0.0762	0	-0.0284	0.1403	0.1365	0.1358	0.0878
DV	-0.0954	0	0.0308	0.1426	0.2016	0.09	0.1685
MN	0.0979	0	-0.1141	0.1064	0.1231	0.0533	0.0478
TA	-0.1985	0	-0.0162	0.3781	0.1253	0.4675	-0.1393
CM	-0.5313	0	0.1243	0.3483	0.4622	0.2534	0.1153
DV	-0.2863	0	0.027	-0.1207	0.1412	-0.0788	-0.0495
CM	-0.145	0	-0.0306	0.0654	0.0316	0.0887	0.0721
OD	-0.0034	0	0.1334	0.073	0.0834	0.0668	0.073
MN	-0.212	0	-0.1407	-0.0147	0.034	-0.0289	0.034
CM	-0.3765	0	-0.0614	0.009	-0.1952	0.0902	-0.0764
OD	-0.0577	0	-0.1025	0.2429	0.1775	0.3281	0.2439
CP	0.1097	0	-0.1503	0.7504	0.914	0.4001	0.4665
DS	N/A	0	0.0231	0.0787	0.0975	0.2495	0.1575
DS	N/A	0	0.0559	0.0591	0.1614	0.1019	0.1905
OD	N/A	0	0.0108	0.2159	0.1538	0.3071	0.2196
CM	N/A	0	-0.0526	-0.0274	-0.1221	0.1299	0.1811
OD	N/A	0	-0.0498	0.4166	0.4237	0.2087	0.349
CM	N/A	0	-0.1719	0.0868	-0.2841	0.1896	0.1251
TA	N/A	0	0.0965	-0.7057	-0.6948	-0.3037	0.0726
AE	N/A	0	-0.0004	-0.0878	-0.0207	0.1072	0.1819
CM	N/A	0	0.1168	0.1115	0.1491	0.0677	0.1683
AE	N/A	0	-0.0006	-0.3559	-0.0836	-0.0448	0.0339
SH	N/A	0	-0.179	-0.4503	-0.0192	0.0807	-0.0181
AE	N/A	0	-0.0715	0.1772	-0.0808	0.2263	-0.0986
CM	N/A	0	0.075	-0.333	-0.5157	-0.0051	-0.0198
TA	N/A	0	0.098	-0.1694	-0.1694	-0.0247	0.093
CM	N/A	0	0.0153	0.0742	-0.0225	0.1615	0.1806
OD	-0.0898	0	0.036	0.5982	0.5388	0.4461	0.4968
DS	-0.2227	0	0.0201	0.235	0.4346	0.1438	0.2298
TS	-0.16	0	0.0047	-0.0494	-0.0363	-0.061	-0.0167
AE	-0.153	0	0.1404	-0.7265	-0.7265	-0.3149	-0.3843
CP	N/A	0	0.0804	0.216	0.2633	0.0403	0.1576

Phase 2

Deoxygenated Hemoglobin (tHb)

SUBJECT	Change Relative to Control (v.d.)					
	0.21 (ata) PIO2	0.21 (ata) PIO2 60 (torr) PETCO2	1.75 (ata) PIO2	1.75 (ata) PIO2 60 (torr) PETCO2	2.80 (ata) PIO2	2.80 (ata) PIO2 60 (torr) PETCO2
OD	0	-0.0983	-0.1689	-0.2363	-0.2414	-0.2983
DD	0	-0.0222	-0.1195	-0.2238	-0.1459	-0.2131
JN	0	-0.0719	-0.1652	-0.2522	-0.1555	-0.2215
MR	0	-0.0910	-0.1230	-0.2715	-0.1284	-0.2544
WW	0	-0.0125	-0.1378	-0.1979	-0.0156	-0.2075
OD	0	-0.0098	-0.1461	-0.1828	-0.1451	-0.1736
CM	0	-0.0654	-0.0373	-0.1333	-0.1191	-0.2796
TA	0	-0.0246	-0.1234	-0.1787	-0.0758	-0.1570
DV	0	-0.1156	-0.1473	-0.2244	-0.1583	-0.2595
BW	0	-0.0164	-0.0243	-0.0626	-0.0357	-0.0578
OD4	0	-0.0482	0.0308	-0.0421	0.0044	-0.0295
CM2	0	-0.0355	-0.1789	-0.2924	-0.1890	-0.3229
DD2	0	-0.0255	-0.1098	-0.2189	-0.2019	-0.2511
DV2	0	-0.0481	-0.1737	-0.2919	-0.1973	-0.2989
JN2	0	-0.1481	-0.0801	-0.2607	-0.1695	-0.3121
TA2	0	-0.0611	-0.0741	-0.1673	-0.0151	-0.1451
MR2	0	-0.0782	-0.1137	-0.2727	-0.2027	-0.3393
WW2	0	-0.0216	-0.1948	-0.2427	-0.2017	-0.2474
PF	0	-0.1091	-0.0817	-0.2270	-0.1235	-0.2580
PF2	0	-0.1068	-0.0817	-0.2035	-0.0702	-0.3057
BW2	0	-0.0200	-0.0272	-0.0687	-0.0763	-0.1330
PF3	0	-0.2160	-0.0944	-0.3095	-0.1885	-0.3425
OD5	0	-0.0984	-0.1505	-0.2793	-0.0578	-0.1770
BC	0	-0.1809	-0.1341	-0.3247	-0.1807	-0.2978
PF4	0	-0.1582	-0.1664	-0.3671	-0.2391	-0.4315
BC2	0	-0.1118	-0.0158	-0.1418	-0.0722	-0.1763
WET DATA						
AVG	0	-0.0696	-0.1121	-0.2058	-0.1233	-0.2335
SD	0	0.0486	0.0547	0.0738	0.0693	0.0898
W SYM						
AVG	0	-0.0822	-0.1150	-0.2270	-0.1138	-0.2321
SD	0	0.0699	0.0498	0.0927	0.0803	0.1183
W/O SYM						
AVG	0	-0.0739	-0.1061	-0.2136	-0.1398	-0.2413
SD	0	0.0493	0.0631	0.0763	0.0676	0.0763
DRY DATA						
AVG	0	-0.0839	-0.1063	-0.2306	-0.1384	-0.2427
SD	0	0.0637	0.0632	0.0884	0.0762	0.0950
ALL DATA						
AVG	0	-0.0767	-0.1092	-0.2182	-0.1308	-0.2381
SD	0	0.0560	0.0580	0.0808	0.0718	0.0907

Phase 2
Oxygenated Hemoglobin (tHbO2)

SUBJECT	Change Relative to Control (v.d.)					
	0.21 (ata) PIO2	0.21 (ata) PIO2 60 (torr) PETCO2	1.75 (ata) PIO2	1.75 (ata) PIO2 60 (torr) PETCO2	2.80 (ata) PIO2	2.80 (ata) PIO2 60 (torr) PETCO2
OD	0	0.1427	0.1994	0.2912	0.2867	0.3558
DD	0	0.0312	0.0938	0.1971	0.0718	0.1412
JN	0	0.0817	0.1735	0.2026	0.0843	0.1097
MR	0	0.1519	0.2372	0.4584	0.174	0.3316
WW	0	0.0081	0.0204	0.1023	-0.0758	0.0793
OD	0	-0.0014	0.239	0.2685	0.1559	0.159
CM	0	0.2007	0.0349	0.2115	0.1584	0.4472
TA	0	0.0445	0.2124	0.2935	0.0923	0.1846
DV	0	0.1504	-0.0031	0.115	0.043	0.1823
BW	0	0.0566	0.0873	0.1573	0.038	0.0831
OD4	0	0.0864	-0.0609	-0.0076	-0.1563	-0.1058
CM2	0	0.1046	0.0945	0.2866	0.1086	0.2899
DD2	0	0.0287	0.2052	0.3213	0.2645	0.2974
DV2	0	0.0683	0.0854	0.2114	0.0637	0.178
JN2	0	0.1491	0.0728	0.2674	0.1604	0.2946
TA2	0	0.0871	0.1079	0.2628	0.0015	0.1335
MR2	0	0.1362	0.1485	0.3747	0.1612	0.368
WW2	0	0.0452	-0.1038	-0.0219	-0.1327	-0.0657
PF	0	0.1794	0.1196	0.3047	0.2281	0.3814
PF2	0	0.134	0.1346	0.2715	0.1566	0.2673
BW2	0	0.0418	0.0415	0.1025	0.1384	0.1801
PF3	0	0.1593	0.0497	0.2063	0.134	0.2426
OD5	0	0.0886	0.1311	0.2488	0.0351	0.1444
BC	0	0.1608	0.1447	0.3142	0.193	0.2961
PF4	0	0.1323	0.1319	0.3073	0.2044	0.3709
BC2	0	0.0964	-0.0033	0.1063	0.0504	0.1449
WET DATA						
AVERAGE	0.0000	0.0945	0.1198	0.2294	0.1108	0.2198
ST. DEV.	0.0000	0.0631	0.0891	0.1006	0.0918	0.1213
W SYM						
AVERAGE	0.0000	0.0855	0.1310	0.2475	0.0979	0.1959
ST. DEV.	0.0000	0.0505	0.0607	0.0475	0.0684	0.0859
W/O SYM						
AVERAGE	0.0000	0.1056	0.0832	0.2133	0.1034	0.2193
ST. DEV.	0.0000	0.0589	0.0964	0.1325	0.1280	0.1556
DRY DATA						
AVERAGE	0.0000	0.1027	0.0797	0.2209	0.0923	0.2027
ST. DEV.	0.0000	0.0501	0.0846	0.1238	0.1280	0.1503
ALL DATA						
AVERAGE	0.0000	0.0986	0.0998	0.2251	0.1015	0.2112
ST. DEV.	0.0000	0.0560	0.0875	0.1106	0.1095	0.1341

BLOOD VOLUME (tBV)

SUBJECT	Change Relative to Control (v.d.)					
	0.21 (ata) PIO2	0.21 (ata) PIO2 60 (torr) PETCO2	1.75 (ata) PIO2	1.75 (ata) PIO2 60 (torr) PETCO2	2.80 (ata) PIO2	2.80 (ata) PIO2 60 (torr) PETCO2
OD	0	0.0442	0.0304	0.0547	0.0452	0.0574
DD	0	0.009	-0.0256	-0.0268	-0.0741	-0.0718
JN	0	0.0097	0.0082	-0.0497	-0.0712	-0.1117
MR	0	0.0608	0.1142	0.1867	0.0455	0.0772
WW	0	-0.0045	-0.1174	-0.0957	-0.0913	-0.1283
OD	0	-0.0112	0.0931	0.0859	0.0108	-0.0146
CM	0	0.1354	-0.0024	0.0783	0.0393	0.1676
TA	0	0.0198	0.0889	0.1147	0.0163	0.0275
DV	0	0.0349	-0.1503	-0.1094	-0.1152	-0.0771
BW	0	0.0402	0.0631	0.0949	0.0023	0.0253
OD4	0	0.0383	-0.0301	-0.0496	-0.1517	-0.1351
CM2	0	0.0691	-0.0844	-0.0059	-0.0803	-0.033
DD2	0	0.0031	0.0944	0.1022	0.0895	0.0462
DV2	0	0.0203	-0.0881	-0.0123	-0.1334	-0.1207
JN2	0	0.001	0.0067	-0.0073	-0.0091	-0.0176
TA2	0	0.0258	0.0337	0.0954	-0.0137	-0.0117
MR2	0	0.058	0.0348	0.102	-0.0415	0.0286
WW2	0	0.0236	-0.2985	-0.2646	-0.3342	-0.3131
PF	0	0.0705	0.038	0.0777	0.1047	0.1235
PF2	0	0.0272	0.0528	0.068	0.0864	-0.0385
BW2	0	0.0758	0.0682	0.0877	0.116	0.1011
PF3	0	-0.0566	-0.0446	-0.103	-0.0544	-0.0998
OD5	0	-0.01	-0.0195	-0.0306	-0.0229	-0.0327
BC	0	-0.02	0.0106	-0.0104	0.0124	-0.0017
PF4	0	-0.0259	-0.0344	-0.0596	-0.0347	-0.0605
BC2	0	-0.0154	-0.0191	-0.0354	-0.0218	-0.0313
WET DATA						
AVERAGE	0	0.0249	0.0078	0.0236	-0.0125	-0.0138
ST. DEV.	0	0.0419	0.0791	0.0913	0.0615	0.0830
W SYM						
AVERAGE	0	0.0033	0.0161	0.0282	-0.0159	-0.0362
ST. DEV.	0	0.0313	0.0647	0.0801	0.0594	0.0509
W/O SYM						
AVERAGE	0	0.0349	-0.0190	0.0020	-0.0316	-0.0188
ST. DEV.	0	0.0405	0.0996	0.1051	0.1109	0.1169
DRY DATA						
AVERAGE	0	0.0230	-0.0214	-0.0014	-0.0399	-0.0358
ST. DEV.	0	0.0396	0.0995	0.1033	0.1212	0.1137
ALL DATA						
AVERAGE	0	0.0240	-0.0068	0.0111	-0.0262	-0.0248
ST. DEV.	0	0.0400	0.0893	0.0964	0.0952	0.0982

Phase 2
Cytochrome a,a 3 (CYT)

SUBJECT	Change Relative to Control (v.d.)					
	0.21 (ata) PIO2	0.21 (ata) PIO2 60 (torr) PETCO2	1.75 (ata) PIO2	1.75 (ata) PIO2 60 (torr) PETCO2	2.80 (ata) PIO2	2.80 (ata) PIO2 60 (torr) PETCO2
OD	0	0.1303	0.1216	0.155	0.1416	0.1853
DD	0	0.1537	0.0383	0.2279	0.0002	0.0847
JN	0	0.0343	0.4068	0.3581	0.1489	0.1363
MR	0	0.3133	0.3431	0.813	0.2327	0.5147
WW	0	0.0478	-0.3395	-0.1788	-0.3354	-0.2101
OD	0	0.0523	0.0569	0.0784	-0.0321	-0.0152
CM	0	0.3046	0.0883	0.3587	0.193	0.5937
TA	0	0.0893	0.5131	0.7144	0.2701	0.467
DV	0	0.2081	0.0484	0.1769	0.0854	0.2466
BW	0	0.3244	0.4208	0.645	0.2393	0.5187
OD4	0	0.0779	-0.1005	-0.0283	-0.0808	-0.0603
CM2	0	0.1879	0.3342	0.6957	0.2749	0.5314
DD2	0	0.0806	0.3289	0.4429	0.4152	0.3783
DV2	0	0.1024	0.2352	0.3695	0.1523	0.3703
JN2	0	0.1648	0.1695	0.3353	0.1368	0.3084
TA2	0	0.1093	0.0284	0.1988	-0.1012	0.0674
MR2	0	0.2168	0.0514	0.2893	0.1427	0.3277
WW2	0	0.0728	-0.0913	-0.0095	-0.1323	-0.0628
PF	0	0.1842	0.1547	0.3581	0.1631	0.3385
PF2	0	0.2499	0.3044	0.4626	0.274	0.4207
BW2	0	0.187	0.317	0.4702	0.4068	0.5086
PF3	0	0.2786	0.1905	0.4473	0.1981	0.3885
OD5	0	0.0502	0.1431	0.2	0.1399	0.1866
BC	0	0.1056	-0.0686	0.0296	-0.0707	0.0035
PF4	0	0.1872	0.3402	0.4335	0.3217	0.4123
BC2	0	0.0918	0.0724	0.1498	0.088	0.1236

WET DATA						
AVERAGE	0	0.1682	0.1858	0.3380	0.1252	0.2676
ST. DEV.	0	0.1050	0.2298	0.2782	0.1749	0.2418

W SYM						
AVERAGE	0	0.1604	0.2481	0.3944	0.1625	0.3129
ST. DEV.	0	0.1027	0.1624	0.2094	0.1434	0.1872

W/O SYM						
AVERAGE	0	0.1507	0.1103	0.2732	0.1065	0.2322
ST. DEV.	0	0.0822	0.1972	0.2547	0.1898	0.2358

DRY DATA						
AVERAGE	0	0.1399	0.1302	0.2922	0.1265	0.2528
ST. DEV.	0	0.0681	0.1558	0.2108	0.1808	0.2053

ALL DATA						
AVERAGE	0	0.1540	0.1580	0.3151	0.1259	0.2602
ST. DEV.	0	0.0879	0.1944	0.2429	0.1743	0.2199

Phase 3
Cerebral Oxygenation

Subject	0.21 (ata) PIO2			1.75 (ata) PIO2		2.80 (ata) PIO2	
	Control	Start PETCO2 = 50 torr	End PETCO2 = 50 torr	Start PETCO2 = 50 torr	End PETCO2 = 50 torr	Start PETCO2 = 50 torr	End PETCO2 = 50 torr
	tHb - Change from Control (v.d.)						
DD	0	0.001	-0.0659	-0.2799	-0.3706	-0.1955	-0.2744
FC	0	-0.0412	-0.0817	-0.1612	-0.2273	-0.2106	-0.2972
AW	0	-0.0578	-0.126	-0.2271	-0.3327	-0.1721	-0.3143
CI	0	-0.0578	-0.1245	-0.0769	-0.1697	-0.159	-0.203
KS	0	-0.0772	-0.1045	-0.0582	-0.1301	-0.3359	-0.4375
BB	0	-0.1199	-0.1887	-0.2443	-0.3139	-0.2572	-0.3023
CM	0	-0.0764	-0.1706	-0.1946	-0.2972	-0.1772	-0.347
PF	0	-0.0662	-0.1303	-0.0817	-0.1586	-0.1014	-0.1289
MM	0	-0.1042	-0.1743			0.02	-0.1197
MR	0	-0.1422	-0.2145	-0.2664	-0.3636	-0.4553	-0.4434
	tHbO2 - Change from Control (v.d.)						
DD	0	0.001	-0.0659	-0.2799	-0.3706	-0.1955	-0.2744
FC	0	-0.0412	-0.0817	-0.1612	-0.2273	-0.2106	-0.2972
AW	0	-0.0578	-0.126	-0.2271	-0.3327	-0.1721	-0.3143
CI	0	-0.0578	-0.1245	-0.0769	-0.1697	-0.159	-0.203
KS	0	-0.0772	-0.1045	-0.0582	-0.1301	-0.3359	-0.4375
BB	0	-0.1199	-0.1887	-0.2443	-0.3139	-0.2572	-0.3023
CM	0	-0.0764	-0.1706	-0.1946	-0.2972	-0.1772	-0.347
PF	0	-0.0662	-0.1303	-0.0817	-0.1586	-0.1014	-0.1289
MM	0	-0.1042	-0.1743			0.02	-0.1197
MR	0	-0.1422	-0.2145	-0.2664	-0.3636	-0.4553	-0.4434
	tBV - Change from Control (v.d.)						
DD	0	0.001	-0.0659	-0.2799	-0.3706	-0.1955	-0.2744
FC	0	-0.0412	-0.0817	-0.1612	-0.2273	-0.2106	-0.2972
AW	0	-0.0578	-0.126	-0.2271	-0.3327	-0.1721	-0.3143
CI	0	-0.0578	-0.1245	-0.0769	-0.1697	-0.159	-0.203
KS	0	-0.0772	-0.1045	-0.0582	-0.1301	-0.3359	-0.4375
BB	0	-0.1199	-0.1887	-0.2443	-0.3139	-0.2572	-0.3023
CM	0	-0.0764	-0.1706	-0.1946	-0.2972	-0.1772	-0.347
PF	0	-0.0662	-0.1303	-0.0817	-0.1586	-0.1014	-0.1289
MM	0	-0.1042	-0.1743			0.02	-0.1197
MR	0	-0.1422	-0.2145	-0.2664	-0.3636	-0.4553	-0.4434
	CYT - Change from Control (v.d.)						
DD	0	0.001	-0.0659	-0.2799	-0.3706	-0.1955	-0.2744
FC	0	-0.0412	-0.0817	-0.1612	-0.2273	-0.2106	-0.2972
AW	0	-0.0578	-0.126	-0.2271	-0.3327	-0.1721	-0.3143
CI	0	-0.0578	-0.1245	-0.0769	-0.1697	-0.159	-0.203
KS	0	-0.0772	-0.1045	-0.0582	-0.1301	-0.3359	-0.4375
BB	0	-0.1199	-0.1887	-0.2443	-0.3139	-0.2572	-0.3023
CM	0	-0.0764	-0.1706	-0.1946	-0.2972	-0.1772	-0.347
PF	0	-0.0662	-0.1303	-0.0817	-0.1586	-0.1014	-0.1289
MM	0	-0.1042	-0.1743			0.02	-0.1197
MR	0	-0.1422	-0.2145	-0.2664	-0.3636	-0.4553	-0.4434

Phase 4
Cerebral Oxygenation

Subject	0.21 (ata) PIO2		1.75 (ata) PIO2		0.21 (ata) PIO2		2.80 (ata) PIO2	
	Start	End	Start	End	Start	End	Start	End
	tHb - Change from Control (v.d.)							
AW	0	0.0003	-0.1005	-0.1369	0	0.0003	-0.0641	-0.1436
BC	0	0.0008	-0.1571	-0.1882	0	0.0081	-0.0965	-0.1131
BFC	0	0.0036	-0.1106	-0.2592	0	0.0024	-0.1426	-0.183
CI	0	0.0004	-0.0106	-0.1267	0	-0.0061	0.1736	0.1837
CM	0	0.006	-0.1	-0.1076	0	-0.0019	-0.1271	-0.2173
CW	0	0.007	-0.132	-0.2626	0	0.0031	-0.2992	-0.1811
FC	0	-0.0006	-0.0974	-0.1157	0	0.0014	-0.1466	-0.1746
KS	0	0.0118	-0.0992	-0.165	0	0.0031	-0.1272	-0.1887
PF	0	-0.0199	-0.1605	-0.1866	0	0.0343	-0.1315	-0.2221
RS	0	-0.0015	-0.0829	-0.1543	0	-0.0142	-0.1504	-0.1804
RT	0	-0.0019	-0.0831	-0.1149	0	0.0043	-0.0611	-0.0933
	tHbO2 - Change from Control (v.d.)							
AW	0	0.0037	0.0019	0.1076	0	0.0254	-0.0018	0.0638
BC	0	0.0076	0.2504	0.2542	0	0.0025	-0.0179	-0.0317
BFC	0	0.0033	0.1378	0.3642	0	-0.0045	0.3731	0.3351
CI	0	-0.0092	-0.202	-0.0197	0	-0.0129	-0.1756	-0.2419
CM	0	-0.011	0.0423	0.0455	0	-0.0089	0.0951	0.24
CW	0	-0.0069	0.2827	0.4767	0	0.0019	0.4198	0.1216
FC	0	-0.0032	0.0726	0.0892	0	-0.0096	0.1108	0.1236
KS	0	0.0007	0.0115	-0.0389	0	0.0247	0.0996	0.1454
PF	0	0.0302	0.1716	0.2332	0	-0.0389	0.1556	0.2253
RS	0	-0.0235	0.0637	0.1044	0	-0.0101	0.0607	0.0199
RT	0	0.0178	0.0782	0.0837	0	-0.002	-0.0611	-0.0712
	tBV - Change from Control (v.d.)							
AW	0	0.0039	-0.0987	-0.0295	0	0.0257	-0.066	-0.0799
BC	0	0.0084	0.0932	0.066	0	0.0105	-0.1144	-0.1448
BFC	0	0.0068	0.0271	0.1048	0	-0.0022	0.2303	0.152
CI	0	-0.0007	-0.2126	-0.1464	0	-0.019	-0.0019	-0.058
CM	0	-0.005	-0.0576	-0.0621	0	-0.0107	-0.032	0.0227
CW	0	0.0002	0.1507	0.2141	0	0.0112	0.1232	-0.0595
FC	0	-0.0038	-0.0247	-0.0264	0	-0.0082	-0.0359	-0.051
KS	0	0.0125	-0.0877	-0.2039	0	0.0278	-0.0277	-0.0433
PF	0	0.0104	0.011	0.0465	0	-0.0047	0.0239	0.003
RS	0	-0.025	-0.0193	-0.0499	0	-0.0243	-0.0897	-0.1606
RT	0	0.0157	-0.005	-0.0314	0	0.0022	-0.1223	-0.1644
	CYT - Change from Control (v.d.)							
AW	0	-0.009	0.1336	0.3093	0	-0.0111	-0.0715	-0.0021
BC	0	-0.0081	0.2135	0.2926	0	0.0181	-0.0301	-0.0252
BFC	0	0.0023	0.1371	0.0878	0	-0.003	0.3798	0.377
CI	0	-0.0019	0.1649	0.1815	0	0.0403	-0.0763	-0.1547
CM	0	-0.0083	0.0918	0.1452	0	-0.0412	0.1008	0.0044
CW	0	-0.0162	-0.0618	-0.2324	0	0.0088	-0.0681	0.2133
FC	0	-0.0163	0.1089	0.1436	0	-0.061	0.022	0.036
KS	0	0.0313	0.0526	0.0892	0	-0.0232	0.0596	0.1075
PF	0	0.025	0.1821	0.3171	0	-0.0498	0.1709	0.2422
RS	0	0.0342	0.0775	0.1411	0	-0.0262	0.0779	0.1177
RT	0	-0.0015	-0.0352	0.0375	0	-0.007	0.034	0.0192

Phase 5
Cerebral Oxygenation

Phase 5 - Head-out Immersion								
Subject	0.21 (ata) PIO2		1.75 (ata) PIO2		0.21 (ata) PIO2		2.80 (ata) PIO2	
	Start	End	Start	End	Start	End	Start	End
	tHb - Change from Control (v.d.)							
AW	0	-0.0358	-0.1810	-0.2469	0	-0.0431	-0.1226	-0.1619
CM	0	-0.1218	-0.1936	-0.2850	0	-0.0955	-0.2579	-0.2468
CW	0	-0.0321	-0.1918	-0.0175	0	0.0143	-0.2807	-0.3294
KS	0	-0.1034	-0.1848	-0.2658	0	-0.1237	-0.2955	-0.3997
RT	0	0.0117	-0.1195	-0.1729	0	-0.0736	-0.1487	-0.1935
FC	0	-0.0078	-0.1303	-0.1503	0	-0.0158	-0.3159	-0.2860
	tHbO2 - Change from Control (v.d.)							
AW	0	0.0165	0.1418	0.2488	0	0.1396	0.0987	0.1626
CM	0	0.1343	0.2321	0.2985	0	0.1184	0.1731	0.1251
CW	0	0.0636	0.1029	-0.0025	0	-0.0399	0.2402	0.1261
KS	0	0.0001	0.0699	0.1356	0	-0.0016	0.0433	0.0783
RT	0	0.0654	0.2276	0.1693	0	0.0801	0.0953	0.0303
FC	0	-0.0266	0.0653	0.0995	0	-0.1118	0.3244	0.1918
	tBV - Change from Control (v.d.)							
AW	0	-0.0193	-0.0393	0.0018	0	0.0966	-0.0239	0.0008
CM	0	0.0124	0.0384	0.0134	0	0.0229	-0.0849	-0.1218
CW	0	0.0315	-0.0888	-0.0200	0	-0.0256	-0.0405	-0.2034
KS	0	-0.1033	-0.1150	-0.1303	0	-0.1253	-0.2523	-0.3215
RT	0	0.0770	0.1080	-0.0037	0	0.0064	-0.0533	-0.1631
FC	0	-0.0345	-0.0649	-0.0507	0	-0.1312	0.0085	-0.0943
	CYT - Change from Control (v.d.)							
AW	0	-0.0420	0.0976	0.3617	0	0.2269	0.0997	0.2044
CM	0	0.1790	0.2663	0.3529	0	0.1435	0.1709	0.1688
CW	0	0.0084	0.0116	-0.0084	0	0.0389	0.0839	0.1399
KS	0	0.0120	-0.0810	0.0080	0	-0.0320	-0.0760	-0.0310
RT	0	0.1719	0.2251	0.1092	0	0.0627	0.0431	0.0386
FC	0	-0.0090	0.1050	0.1493	0	-0.0425	0.1260	0.0863

Phase 6
Cerebral Oxygenation

Phase 6 - Total Immersion								
Subject	0.21 (ata) PIO2		1.75 (ata) PIO2		0.21 (ata) PIO2		2.80 (ata) PIO2	
	Start	End	Start	End	Start	End	Start	End
	tHb - Change from Control (v.d.)							
FC	0	0.0056	-0.0749	-0.1161	0	-0.1612	-0.1638	-0.1326
CI	0	-0.0011	-0.1637	-0.1542	0	-0.0460	-0.2161	-0.2842
HM	0	0.0198	-0.1745	-0.1966	0	-0.0152	-0.1600	-0.1546
PF	0	-0.1204	-0.2042	-0.2713	0	-0.1322	-0.2622	-0.3607
RS	0	0.1368	0.0616	-0.0822	0	-0.1606	-0.1644	-0.2490
MR	0	-0.0502	-0.2943	-0.1553	0	-0.0449	-0.1948	-0.2597
	tHbO2 - Change from Control (v.d.)							
FC	0	0.0032	0.1823	0.1910	0	0.3544	0.0892	-0.0288
CI	0	0.0024	0.1024	0.1082	0	0.1274	0.1371	0.2951
HM	0	0.1045	0.2165	0.1474	0	0.0431	0.0759	-0.0447
PF	0	0.2033	0.2164	0.2997	0	0.2810	0.3583	0.4496
RS	0	-0.1195	-0.0895	0.0303	0	0.3430	0.0580	0.1400
MR	0	0.0192	0.1603	0.0478	0	0.1115	0.2672	0.3708
	tBV - Change from Control (v.d.)							
FC	0	0.0088	0.1066	0.0748	0	0.1933	-0.0745	-0.1614
CI	0	0.0013	-0.0614	-0.0461	0	0.0814	-0.0791	0.0108
HM	0	0.1244	0.0421	-0.0492	0	0.0279	-0.0842	-0.1995
PF	0	0.0828	0.0121	0.0283	0	0.1488	0.0960	0.0887
RS	0	0.0172	-0.0280	-0.0519	0	0.1824	-0.1065	-0.1091
MR	0	-0.0309	-0.1339	-0.1075	0	0.0666	0.0726	0.1111
	CYT - Change from Control (v.d.)							
FC	0	-0.0400	0.1110	0.1168	0	0.2397	0.4030	0.3698
CI	0	-0.0130	0.3574	0.0807	0	-0.0600	-0.0100	0.0410
HM	0	0.1733	0.2760	0.2807	0	-0.0276	0.0684	0.0667
PF	0	0.1661	0.1819	0.3008	0	0.2360	0.2866	0.3800
RS	0	0.4102	0.8321	0.4924	0	0.1769	0.1593	0.2170
MR	0	-0.1569	-0.2067	0.3083	0	-0.0921	0.0076	0.0535

Phase 7
Cerebral Oxygenation

Phase 7 - Total vs Head-out Immersion						
Subject	0.21 (ata) PIO2			2.80 (ata) PIO2		
	HO Immersion	Total Immersion	HO Immersion	HO Immersion	Total Immersion	HO Immersion
	tHb - Change from Control (v.d.)					
FC	0	-0.0574	-0.0381	-0.0855	-0.1397	
HM	0	-0.0487		-0.1158	-0.0032	
BL	0	0.0153	0.0110	-0.0799	-0.1319	
JB	0	-0.0186		-0.0600	-0.1896	
MR	0	-0.0063		-0.2193	-0.2303	
RT	0	0.0491		-0.1364	-0.1541	
RF	0	-0.0597	0.0020	-0.2102	-0.3186	-0.2794
RS	0	0.0111	0.0079	-0.1150	-0.1326	-0.1523
JH	0	-0.0261	0.0117	-0.2646	-0.4036	
DL	0	-0.1059	-0.0080	-0.1409	-0.0190	
	tHbO2 - Change from Control (v.d.)					
FC	0	0.2880	0.0482	-0.0254	0.1712	
HM	0	0.3191		-0.0020	-0.1093	
BL	0	0.1012	0.0318	0.0035	0.1635	
JB	0	0.1559		0.0564	0.3461	
MR	0	0.1360		0.1077	0.1905	
RT	0	0.2460		-0.0440	0.1989	
RF	0	0.2327	-0.0311	0.1356	0.4052	0.2440
RS	0	0.0043	0.0226	0.0019	0.0981	0.0167
JH	0	0.1351	0.0153	0.2532	0.5360	
DL	0	0.4574	0.0201	0.3701	0.2759	
	tBV - Change from Control (v.d.)					
FC	0	0.2307	0.0101	-0.1110	0.0315	
HM	0	0.2705		-0.1178	-0.1126	
BL	0	0.1166	0.0430	-0.0762	0.0316	
JB	0	0.1374		-0.0036	0.1565	
MR	0	0.1298		-0.1115	-0.0397	
RT	0	0.2950		-0.1806	0.0446	
RF	0	0.1729	-0.0292	-0.0747	0.0865	-0.0355
RS	0	0.0155	0.0305	-0.1129	-0.0344	-0.1354
JH	0	0.1090	0.0270	-0.0115	0.1322	
DL	0	0.3515	0.0327	0.0451	0.2568	
	CYT - Change from Control (v.d.)					
FC	0	0.1877	0.0464	0.0096	0.1436	
HM	0	0.2217		0.2873	0.8524	
BL	0	0.0647	0.0411	0.0200	0.1240	
JB	0	0.0332		0.0484	0.1529	
MR	0	0.1915		0.0252	0.1114	
RT	0	0.2452		0.1249	0.4324	
RF	0	0.4234	-0.0057	0.0752	0.4157	0.2017
RS	0	0.0575	0.0535	0.0455	0.1504	0.0978
JH	0	0.1902	0.0401	0.2660	0.5037	
DL	0	0.4836	0.0781	0.2855	0.1864	

Phase 8
Cerebral Oxygenation

Phase 8 - Total vs Head-out Immersion with Positive SLL							
Subject	0.21 (ata) PIO2						
	H0 Immersion	HO Immersion + BH	Total Immersion	H0 Immersion Recovery	Total Immersion 2	Total Immersion + BH	HO Immersion Recovery 2
	tHb - Change from Control (v.d.)						
TA	0	-0.0086	-0.0497	0.0033	0.0042	-0.0079	-0.0030
FC	0	-0.0139	-0.0456	0.0006	-0.0208	-0.0250	0.0000
RS	0	0.0215	-0.0046	0.0040	0.0070	0.0229	0.0086
JB	0	0.0058	-0.0581	0.0066	-0.0586	-0.0636	-0.0002
CI	0	-0.0080	-0.0451	-0.0490	-0.0400	-0.0561	-0.0747
HM	0	0.0055	-0.0017	0.0285	0.0152	0.0015	0.0238
	tHbO2 - Change from Control (v.d.)						
TA	0	0.0232	0.1714	-0.1112	0.1593	0.2147	-0.0189
FC	0	0.0287	0.0611	-0.0316	0.0481	0.0644	0.0000
RS	0	0.0132	0.0192	-0.0274	0.0093	0.0151	-0.0064
JB	0	-0.0055	0.2415	-0.0554	0.2097	0.2626	-0.0425
CI	0	0.0061	0.0608	0.0479	0.0570	0.0910	0.0895
HM	0	0.0081	0.0368	-0.0511	0.0049	0.0756	-0.0151
	tBV - Change from Control (v.d.)						
TA	0	0.0145	0.1217	-0.1080	0.1635	0.2103	-0.0220
FC	0	0.0146	0.0154	-0.0311	0.0272	0.0394	0.0000
RS	0	0.0347	0.0146	-0.0264	0.0163	0.0381	0.0023
JB	0	0.0003	0.1834	-0.0489	0.1511	0.1990	-0.0428
CI	0	-0.0019	0.0156	-0.0011	0.0169	0.0349	0.0148
HM	0	0.0136	0.0352	-0.0225	0.0201	0.0771	0.0087
	CYT - Change from Control (v.d.)						
TA	0	0.0508	0.1899	0.0229	0.2121	0.3118	0.0839
FC	0	0.0074	0.0427	-0.0333	0.0292	0.0609	0.0000
RS	0	0.0112	0.0380	0.0000	0.0779	0.0871	0.0623
JB	0	0.0245	0.1321	-0.1680	0.0876	0.1386	-0.1225
CI	0	0.0030	0.0173	0.0132	0.0218	0.0977	0.0664
HM	0	0.0250	0.0496	-0.0315	0.0213	0.1500	0.0238

Phases 9 and 10
Cerebral Oxygenation

Phase 9 - Dry Work					Phase 10 - Total Immersion - Work					
Sub.	0.21 (ata) PIO2		1.75 (ata) PIO2		Sub.	0.21 (ata) PIO2			1.75 (ata) PIO2	
	Rest	Work	Rest	Work		Dry Rest	Rest	Work	Rest	Work
tHb - Change from Control (v.d.)					**tHb - Change from Control (v.d.)**					
BP	0	-0.1255	-0.0585	-0.2635	CI	0	-0.0770	-0.0691	-0.1067	-0.4863
CI	0	-0.0124	-0.1023	-0.2897	FC	0	-0.0211	-0.0299	-0.3001	-0.2728
FC	0	-0.0040	-0.0945	-0.1592	JB	0	-0.0940	-0.0132	0.0484	-0.0993
HM	0	-0.0978	-0.1254	-0.1638	KB	0	-0.3336	-0.2943	-0.6354	-0.6453
KB	0	-0.3550	-0.0620	-0.3223	PD	0	-0.0700	-0.0139	-0.1392	-0.2818
KS	0	0.0616	-0.0129	-0.1456	PF	0	-0.0405	0.0118	-0.0034	-0.3616
PF	0	-0.1176	-0.1472	-0.6025	RF	0	-0.1013	-0.0180	-0.2755	-0.4636
RS	0	0.0179	-0.1500	-0.1984	RS	0	-0.4250	-0.4876	-0.0887	-0.0676
RT	0	-0.1070	-0.0072	-0.1107	RT	0	-0.0199	0.0258	-0.0595	-0.1302
tHbO2 - Change from Control (v.d.)					**tHbO2 - Change from Control (v.d.)**					
BP	0	0.2883	0.0420	0.4588	CI	0	0.0586	0.0098	0.0487	0.6080
CI	0	-0.0037	0.1956	0.4625	FC	0	0.0594	0.0600	0.5870	0.4561
FC	0	0.0321	0.1746	0.2916	JB	0	0.2922	0.0468	-0.0192	0.1764
HM	0	0.2418	0.0480	0.0596	KB	0	0.3988	0.1552	0.5887	0.6720
KB	0	0.6227	-0.0870	0.2895	PD	0	-0.0040	-0.1771	-0.2922	-0.1219
KS	0	-0.0687	0.0551	0.1258	PF	0	0.0673	0.0604	0.0113	0.4450
PF	0	0.1232	0.1233	0.8593	RF	0	0.1611	0.0410	0.1125	0.3246
RS	0	-0.0229	0.0736	0.0325	RS	0	0.4987	0.6405	0.0510	0.0242
RT	0	0.2627	-0.1319	-0.0606	RT	0	0.0562	-0.0502	0.1145	0.1200
tBV - Change from Control (v.d.)					**tBV - Change from Control (v.d.)**					
BP	0	0.1627	-0.0166	0.1951	CI	0	-0.0184	-0.0593	-0.0580	0.1216
CI	0	-0.0161	0.0969	0.1729	FC	0	0.0383	0.0300	0.2867	0.1831
FC	0	0.0283	0.0802	0.1324	JB	0	0.1983	0.0336	0.0292	0.0771
HM	0	0.1441	-0.0773	-0.1042	KB	0	0.0653	-0.1390	-0.0467	0.0267
KB	0	0.2676	-0.1489	-0.0329	PD	0	-0.0740	-0.1910	-0.4314	-0.4038
KS	0	-0.0072	0.0421	-0.0199	PF	0	0.0269	0.0724	0.0017	0.0834
PF	0	0.0057	-0.0239	0.2568	RF	0	0.0598	0.0230	-0.1631	-0.1390
RS	0	-0.0050	-0.0765	-0.1660	RS	0	0.0738	0.1530	-0.0376	-0.0434
RT	0	0.1556	-0.1391	-0.1714	RT	0	0.0363	-0.0244	0.0549	-0.0102
CYT - Change from Control (v.d.)					**CYT - Change from Control (v.d.)**					
BP	0	0.4577	0.0746	0.5310	CI	0	-0.0178	0.0620	0.0946	-0.2550
CI	0	0.0429	-0.0870	0.0853	FC	0	0.1421	0.1339	0.8518	0.6749
FC	0	0.0696	0.2280	0.4287	JB	0	1.0452	0.7191	0.4349	0.7877
HM	0	-0.0148	0.1813	0.1558	KB	0	0.4787	0.7844	-0.1550	0.9605
KB	0	-0.3142	0.0448	0.1526	PD	0	-0.0121	-0.0704	-0.1072	0.0282
KS	0	0.0229	-0.3216	-0.2156	PF	0	0.0212	0.0714	0.0620	0.3501
PF	0	0.2870	0.0320	-0.1950	RF	0	0.2560	0.1194	0.0223	0.2698
RS	0	0.0240	0.1978	0.2066	RS	0	0.8388	1.1127	0.4513	0.3932
RT	0	-0.0404	0.3463	0.3481	RT	0	0.0661	-0.0013	0.3015	0.3698

ABSTRACT

Elevated partial pressures of inspired oxygen and carbon dioxide reduce the time to onset of symptoms of central nervous system (CNS) oxygen toxicity. The effects of hyperoxia and hypercapnia on cerebral oxygenation, ventilatory response, and symptoms of CNS oxygen toxicity were investigated in 11 subjects using a computer controlled, closed-circuit breathing apparatus. Inspired oxygen partial pressure (PI_{O_2}) was maintained at 0.21, 1.75 or 2.80 ATA and minute ventilation was measured during rebreathing as the end-tidal carbon dioxide (PET_{CO_2}) rose from 40 to 60 torr. Relative changes in cerebral oxygenation from normoxia were monitored using near infrared spectroscopy (NIRS), a non-invasive technique for continuous measurement of deoxygenated hemoglobin (Hb), oxygenated hemoglobin (HbO_2), blood volume and cytochrome a,a_3 oxidation-reduction (redox) level. Definite symptoms (tunnel vision, tinnitus, and extreme anxiety) associated with CNS oxygen toxicity were reported within 3 torr of the maximum PET_{CO_2} at 2.80 ATA PI_{O_2} in 7 of 34 experiments. Another 14 minor symptoms (tingling, numbness, narcosis, sweats, and dizziness) possibly due to oxygen toxicity were reported. All subjects with definite

symptoms and 9 of 12 with minor symptoms showed low ventilatory response slopes (ventilation vs. PET_{CO_2}) at 2.80 ATA PI_{O_2} relative to normoxic slopes. Cerebrocortical blood volume relative to normoxia, decreased with increasing PI_{O_2}. CO_2 rebreathing reversed this effect such that relative cortical blood volume was significantly greater ($p < .05$) at a PET_{CO_2} of 60 torr than at normocapnia for each PI_{O_2}. Despite the increase in blood volume with CO_2, the amount of oxidized cytochrome a,a3 did not change significantly. Experiments in which symptoms were reported resulted in smaller blood volume changes at maximum PET_{CO_2} for a PI_{O_2} of 2.80 ATA than at 0.21 ATA PI_{O_2}. In summary, ventilatory response measurements suggest some individuals have depressed CO_2 response during hyperoxia and may be more susceptible to CNS oxygen toxicity. The use of NIRS during hyperoxic hypercapnia shows evidence for normal regulation of the oxidation state of cytochrome a,a3 in the mitochrondria at PI_{O_2} up to 2.80 ATA and PET_{CO_2} up to 60 torr. Symptoms of CNS oxygen toxicity may occur without reversal of hyperoxic cerebral vasoconstriction during CO_2 breathing.

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LIST OF ABBREVIATIONS

ATA	Atmospheres Absolute
BTPS	Body Temperature Pressure Saturated
CNS	Central Nervous System
CYT	Cytochrome a,a_3
fsw	feet of sea water
Hb	Deoxygenated Hemoglobin
HbO_2	Oxygenated Hemoglobin
lpm	liters per minute
NIRS	Near Infrared Spectroscopy
Pa_{CO_2}	Arterial Oxgyen Pressure
P_{CO_2}	Carbon Dioxide partial pressure
PET_{CO_2}	End-Tidal Carbon Dioxide Pressure
PI_{CO_2}	Inspired Carbon Dioxide Pressure
PI_{O_2}	Inspired Oxygen Pressure
P_{O_2}	Oxygen partial pressure
Pt_{CO_2}	Tissue carbon dioxide partial pressure
Pt_{O_2}	Tissue oxygen partial pressure
Pv_{CO_2}	Venous Oxygen Pressure
RMV	Respiratory Minute Volume
Sa_{O_2}	Arterial Oxygen Saturation
STPD	Standard Temperature Pressure Dry
V_T	Tidal Volume

1. INTRODUCTION

Exposure to sufficient amounts of oxygen eventually becomes toxic to cells in all tissues of the body (Clark 1982). In fact, normal oxygen pressure would be toxic to most living creatures in the absence of intracellular antioxidant defenses (Fridovich 1988). The central nervous system (CNS) is acutely susceptible to the toxic effects of high partial pressures of oxygen. Regulatory mechanisms of circulation and a high metabolic rate require hyperbaric oxygen (HBO) pressures ($\geq$ 1.5 ATA O_2) to precipitate CNS oxygen toxicity in man (Lambertsen 1978). At hyperbaric oxygen pressures, oxygen delivery to the brain can exceed tolerable levels and produce signs and symptoms of oxygen toxicity. Given sufficient oxygen pressure, the onset time to the occurrence of symptoms of CNS oxygen toxicity is decreased by exposure duration, inspired carbon dioxide, immersion, and work (Young 1971). Any factor which enhances cerebral oxygenation has the potential to increase the rate at which CNS oxygen toxicity develops. There is a large variability in the susceptibility to CNS oxygen toxicity between subjects and by a single subject over time (Donald 1947). The use of hyperbaric oxygen for an increasing number

of therapeutic purposes and greater demand for the use of hyperoxic gas mixtures to decrease decompression time in diving, will expose many individuals to oxygen pressures capable of inducing CNS oxygen toxicity. Presently, there is no objective method to monitor the development of CNS oxygen toxicity (Fife 1991a).

The present study was designed to evaluate the effect of increased inspired oxygen (PI_{O_2}) and carbon dioxide (PI_{CO_2}) on the development of CNS oxygen toxicity by monitoring cerebral oxygenation, ventilatory response, and the occurrence of symptoms. The hypotheses for this work were 1) as inspired CO_2 increases, oxygen delivery to the brain increases and correlates positively with the likelihood that symptoms of CNS oxygen toxicity will develop, 2) the onset of CNS oxygen toxicity may be predicted by tracking changes in cerebral oxygenation and ventilation, and 3) correlation of physiological responses with the occurrence of oxygen toxicity symptoms may provide a means of determining individual susceptibility to CNS oxygen toxicity.

Near Infrared Spectroscopy (NIRS) was first used to monitor changes in cerebral oxygenation in 1977 (Jobsis 1977). This non-invasive technique for continuous measurement of deoxygenated hemoglobin (Hb), oxygenated hemoglobin (HbO_2), blood volume and cytochrome a,a_3 oxidation-reduction (redox) level is used to assess qualitative trends in regional oxygenation and metabolism

(Piantadosi 1989b). NIRS was used to track relative changes in the amount of cerebral oxygen delivery at three oxygen partial pressures (0.21, 1.75, and 2.80 ATA PI_{O_2}) and increasing carbon dioxide to 60 torr PET_{CO_2}.

Ventilatory response to carbon dioxide has traditionally been used to study the respiratory system. CO_2 is the most commonly used chemical stimulus and ventilation the most commonly measured response utilized to investigate various mechanisms involved in respiratory control (Rebuck and Slusky 1981). Increased inspired oxygen pressures lead to decreased ventilatory response to CO_2 and possibly to carbon dioxide retention (Lambertsen 1978). Individuals that retain CO_2 may be more susceptible to CNS oxygen toxicity (Lanphier 1975).

2. BACKGROUND

CNS Oxygen Toxicity

History

Oxygen is necessary for all higher life forms on earth, yet oxygen can also be toxic. Even its discoverer, Joseph Priestly, in 1774 believed O_2 had toxic properties (Priestley 1775). Paul Bert showed in 1878 that brief hyperbaric oxygen exposures resulted in convulsions and death of animals (Bert 1878). In 1899, Lorraine Smith demonstrated that exposures to oxygen partial pressures as low as 0.75 ATA over prolonged periods result in lethal lung damage (Smith 1899).

It is now known that oxygen becomes toxic to cells in every type of tissue given sufficient concentration and exposure duration. Oxygen in itself is not a toxin but is pharmacologically active and participates in a variety of intracellular reactions which may have toxic effects (Clark 1982). A tissue's susceptibility to the toxic effects of oxygen is determined by its biochemical characteristics, antioxidant defenses, metabolic activities, and oxygen supply (Fife 1991a). Some tissues are effected at atmospheric pressures while hyperbaric pressures are required to produce toxicity in others. The CNS is among the most susceptible of

all tissues. Hyperbaric oxygen exposure, $PI_{O_2} \geq 1.5$ ATA, is required to produce the manifestations of toxicity due to the presence of various protective mechanisms. Regional vascular regulation, multiple concentration gradients, and high rates of oxidative metabolism act to protect the brain at atmospheric pressures by limiting oxygen concentrations in cerebral tissue. It is only at hyperbaric pressures that oxygen concentrations in the brain may approach those experienced by the lung at ambient pressures.

Due to the use of hyperbaric oxygen as a therapeutic treatment for several common diseases and the need to use hyperoxic gas mixtures in diving operations, there is a need to determine the pathology of this phenomenon or find a method to monitor its progression. Much research has been done but there are no definitive answers to the questions addressing the underlying causes of CNS oxygen toxicity or a way to monitor its development.

Clinical Manifestations

Donald performed a unique set of experiments using CNS oxygen toxicity symptoms as an index of oxygen poisoning in human exposures to 3.0 ATA PI_{O_2} or higher. The manifestations of the condition included visual distortion, tinnitus, nausea, facial twitching, irritability, and dizziness, among the 36 different symptoms recorded prior to tonic/clonic seizures, undistinguishable from grand mal seizures.

Symptoms did not always precede seizures and seizures did not always occur. Air breathing terminated the seizures with no residual neurological effect after a brief period of postictal confusion. Oxygen seizures also may resolve spontaneously without decreasing the inspired O_2 and seizures may occur minutes after air breathing has begun, a delayed onset referred to as the "off-effect". Attempts to correlate factors such as age, weight, physical fitness, smoking, alcohol ingestion, psychological stability or personality traits failed (Donald 1947). For patients undergoing hyperbaric oxygen therapy, an oxygen seizure is a less significant hazard than for divers. An in-water seizure could prove disastrous due to the high probability that drowning or air embolism could occur.

Factors Affecting Susceptibility to CNS O_2 Toxicity

Susceptibility to oxygen toxicity is widely variable, making definitive dose-response relationships difficult to formulate although some individuals exhibit unusual susceptibility. Variability in susceptibility exists between subjects and for a single subject from day to day (Donald 1947).

Factors which increase oxygen delivery to the brain, such as elevated PI_{O_2}, longer duration, inspired CO_2, work, and immersion have been found to increase the incidence and decrease the onset time of CNS oxygen toxicity symptoms.

Elevated blood flow due to the release of a normally maintained vasoconstriction is a primary cause of increased oxygen delivery (Torbati 1987). Failure of oxygen induced vasoconstriction, as a result of tissue CO_2 tension increase or ischemic injury (Lambertsen et al.1955; Miller et al. 1970), has been shown to occur before the onset of seizures (Bean, Lingnell, and Burgess 1972). Resulting increases in tissue oxygen tension may produce oxygen levels which precipitate symptoms of CNS oxygen toxicity. Physiologic factors such as prevailing adrenergic tone and endocrinologically mediated stress reactions may alter oxygen seizure thresholds. Factors which increase metabolic rate above normal such as fever, hyperthermia, hyperthyroidism or an increase in catecholamines can increase the risk of oxygen toxicity, while hypothyroidism, starvation, and hypothermia decrease risk (Fife 1991a).

Proposed Mechanisms of CNS Oxygen Toxicity

Hyperbaric oxygen exposure has been shown to cause complex biochemical changes in neuronal tissue including inactivation of intracellular enzymes, increased formation of lipid hydroperoxides, changes in the balance of cerebral neurotransmitters such as GABA depletion, Na^+-K^+ ATPase oxidation, and decreased mitochondrial respiration rate leading to decreased ATP availability. It is now generally accepted that the initial event in the development of oxygen

toxicity is increased production of oxygen free radicals (Yusa, Crapo, and Freeman 1984) which follows from increased oxygen delivery (Gerschman et al. 1954). Free radicals, such as the superoxide anion (O_2^-) are formed as a result of the partial reduction of O_2. Free radicals have a single unpaired electron making them very reactive. During aerobic respiration, oxygen is normally completely reduced to water via the transfer of four electrons from cytochrome a,a_3. However, other normal cellular functions may produce free radical by-products as a result of incomplete reduction of O_2. These reactive oxygen species oxidize enzymes and lipids essential for cellular homeostasis (Freeman and Crapo 1982). Naturally occurring enzymes such as superoxide dismutase, catalytically scavenge O_2^- because it is potentially toxic to cells (Fridovich 1979a). Free radicals also enter into reactions producing other potentially toxic species such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radicals ($OH^\bullet$). Hydrogen peroxide is toxic to cells and can also be reduced to the extremely reactive hydroxyl radical, the strongest oxidizing agent known (Fridovich 1975). The reduction of H_2O_2 by transition metals, such as iron, may be important in the process of tissue injury. The rate of generation of superoxide anions and hydrogen peroxide has been shown to increase at increased oxygen pressures (McCord and Fridovich 1978; Yusa et al. 1987).

The means by which reactive species of oxygen are formed has not been fully characterized in the brain although numerous mechanisms have been proposed by which free radicals mediate oxygen toxicity including effects on cellular structure, respiration and metabolic pathways. Some hyperoxic effects which have been measured *in vitro* are:

1) Peroxidation of lipids causing cell membrane damage (Freeman and Crapo 1982).
2) Inactivation of sulfhydryl groups of vital enzymes and co-factors such as glutathione, lipoic acid and co-enzyme A (Haugaard 1968).
3) Rapid oxidation of reduced pyridine nucleotides thus altering cellular respiration and energy production via oxidative phosphorylation (Chance et al 1966).
4) Inhibition of other critical metabolic enzymes, such as those of the pentose shut which provide NADPH to maintain the supply of reduced glutathione (Balentine 1982).
5) Inhibition of DNA, RNA and protein synthesis (Gilbert et al. 1957).
6) Inactivation of Na^+-K^+ ATPase, thus disrupting membrane transport, ionic balance, and neuronal conduction (Gottlieb, Koehler, and Rhodes 1976).

The exact sites of free radical production have not been found but superoxide anion production has been shown to

increase in mitochondria, microsomes, and endoplasmic reticulum in proportion to increased oxygen pressure. (Yusa et al. 1987). Recently, it has been shown that H_2O_2 production by monoamine oxidase is greatly enhanced in the brain at increased oxygen pressures (Zhang and Piantadosi 1990).

Because reactive species are formed during normal cellular function, natural defense systems must be in place to prevent damage due to free radicals. These defense mechanisms include the following:

1) Superoxide dismutases (SOD) are the primary defense against free radicals. SOD catalyzes the dismutation of the superoxide anion via the reaction:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Further enzymatic defenses are necessary to prevent the accumulation of H_2O_2 .

2) Catalase enzymes react with H_2O_2 to reduce it to water.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

3) Numerous peroxidases act as reductants aided by co-substrates such as glutathione. Glutathione peroxidase is capable of degrading lipid peroxides and H_2O_2 (Cross et al. 1977).

Non-enzymatic defenses exist in the form of various antioxidants such as vitamin E, Alph-tocopherol, which is believed to protect against lipid peroxidation.

Prevention and Monitoring CNS Oxygen Toxicity

Introducing air breaks during treatments with HBO decreases the chance that symptoms and seizures will occur (Fife 1991a). Air breaks, however, are not always practical for divers. The use of prophylactic antiseizure medication has proven successful but may worsen underlying cytosolic processes (Haugaard 1968; Van den Brenk and Jamieson 1964). Protection from the toxic effects of oxygen has been investigated using the following approaches:

1) decreasing metabolic rate, e.g. hypothermia,
2) altering neuronal transmission or excitability, e.g. GABA administration,
3) supplementing critical metabolites, e.g. succinate,
4) enhancing antioxidant defenses, e.g. SOD administration.

Each of these methods has shown some promise in animal models but not all are practical for use in humans and none have been shown to be totally effective (Fife 1991a).

Changes in the level of certain metabolic indicators have been correlated with CNS oxygen toxicity. At 2.0 ATA, cerebral spinal fluid lactate increased considerably. Decreases in ATP

and NADH redox cycling have been shown in hyperbaric exposure. The majority of cellular ATP produced in the brain is utilized by the sodium-potassium ATPase enzyme system to maintain ionic balance across the cell membrane. Extracellular K^+ has been shown to increase prior to and during clinical hyperoxic convulsions (Mayevsky 1984). The concentration of the inhibitory brain neurotransmitter GABA decreases prior to oxygen seizures and returns to normal upon resolution of seizures (Clark 1981). Even if these metabolic changes are proven to indicate the presence or development of CNS oxygen toxicity, none are easily monitored in real time.

EEG changes prior to convulsion have been characterized in animals, but definite preconvulsive EEG signatures have not been found in humans (Torbati 1987). At present there is no objective way to monitor the development of CNS oxygen toxicity (Fife 1991a).

Cerebral Oxygenation

The importance of oxygen to the brain is reflected in the fact that it accounts for 20% of O2 uptake and receives 15% of the cardiac output, yet is only 2-3% of total body weight (Jain 1989a). Cerebral blood flow, cerebrocortical oxygenation, and energy metabolism are affected by the partial pressures of oxygen and carbon dioxide. Monitoring changes in cerebral oxygenation might provide insight into oxygen tolerance and the pathophysiology of toxicity.

Oxygen Supply

Oxygen is carried in blood via physical solution and chemical binding. Normally dissolved oxygen is not a significant contributor (.3 ml /100 ml blood) but can become so at elevated PI_{O_2}'s. At 3 ATA O_2, for example, animals transfused with blood substitute are sustained by dissolved oxygen alone (Boerema et al. 1960). Hemoglobin is the carrier to which oxygen is chemically bound. Under normal conditions, nearly all oxygen is delivered to tissues by reversible chemical binding to hemoglobin. Deoxygenated hemoglobin (Hb) binds oxygen in the capillaries of the alveoli and oxygenated hemoglobin (HbO_2) releases oxygen in the capillaries in tissue. Hb plays a major role in the transport of the CO_2 formed as a by-product of cellular respiration. Hb is a complex protein consisting of four polypeptide chains each with a haem group for bonding oxygen. It has the property of ligand binding such that the ligand affinity of a protein depends on the degree of saturation with ligand. Many factors affect the affinity of hemoglobin for O_2. Decreased affinity for oxygen occurs with decreasing pH, and increasing carbon dioxide tension, temperature, 2,3 diphosoglycerate (DPG) concentration, and metabolism. The major driving force for chemically bound O_2/CO_2 transport is the presence of concentration gradients. These gradients are increased during hyperoxic gas breathing.

The brain is not exposed to the full effects of the elevated oxygen content of blood during hyperbaric exposures. Oxygen has a direct vasoconstrictive effect on the control of vascular smooth muscle in the microcirculation. Cellular oxygen demands may regulate blood flow and thereby affect tissue oxygenation. (Torbati 1987). The brain has a high oxidative metabolic rate that requires a continuous and closely matched blood flow.

CO_2 Effect

Dissolved CO_2 is the humoral stimulus for respiration. It is also a powerful cerebral vasodilator (Lambertsen 1978). When faced with hyperoxic blood, the brain protects itself via vasoconstriction, which decreases blood flow and therefore oxygen delivery. Increased CO_2 has been shown to release this protective mechanism. In men breathing 3.5 ATA PI_{O_2}, internal jugular venous P_{O_2} is increased from less than 100 torr to about 1000 torr by elevating PI_{CO_2} from 0 to about 53 torr (Lambertsen et al. 1955). Higher PI_{CO_2}'s increase cerebral blood flow and thus oxygenation, increasing the risk of developing CNS oxygen toxicity.

Oxygen Utilization

The importance of oxygen is its vital role in cellular respiration. High energy phosphates, ATP, are necessary for cellular function. Nearly all ATP in brain tissue is

produced by mitochondrial electron transport. Oxygen is the final electron acceptor in the respiratory chain. Oxygen reacts with a high affinity site of the cytochrome a,a_3 complex, or cytochrome c oxidase, to form water in a four electron process.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O + \text{free energy}$$

To measure oxygen utilization, it would be most useful to determine the oxidation-reduction (redox) state of cytochrome c oxidase because it reduces 90% of the oxygen consumed by the brain tissue during aerobic respiration. Oxidation-reduction reactions are chemical reactions in which electrons are transferred from one molecule to another, therefore, the redox state of a molecule is a measure of reaction progress. The redox state of cytochrome a,a_3 is an important indicator of energy provision during pathologic states characterized by disordered O_2 delivery such as hypoxia (Piantadosi 1989b). Figure 1 shows a simplified diagram of the mitochondrial respiratory chain.

Schematic of Mitochrondrial Respiratory Chain

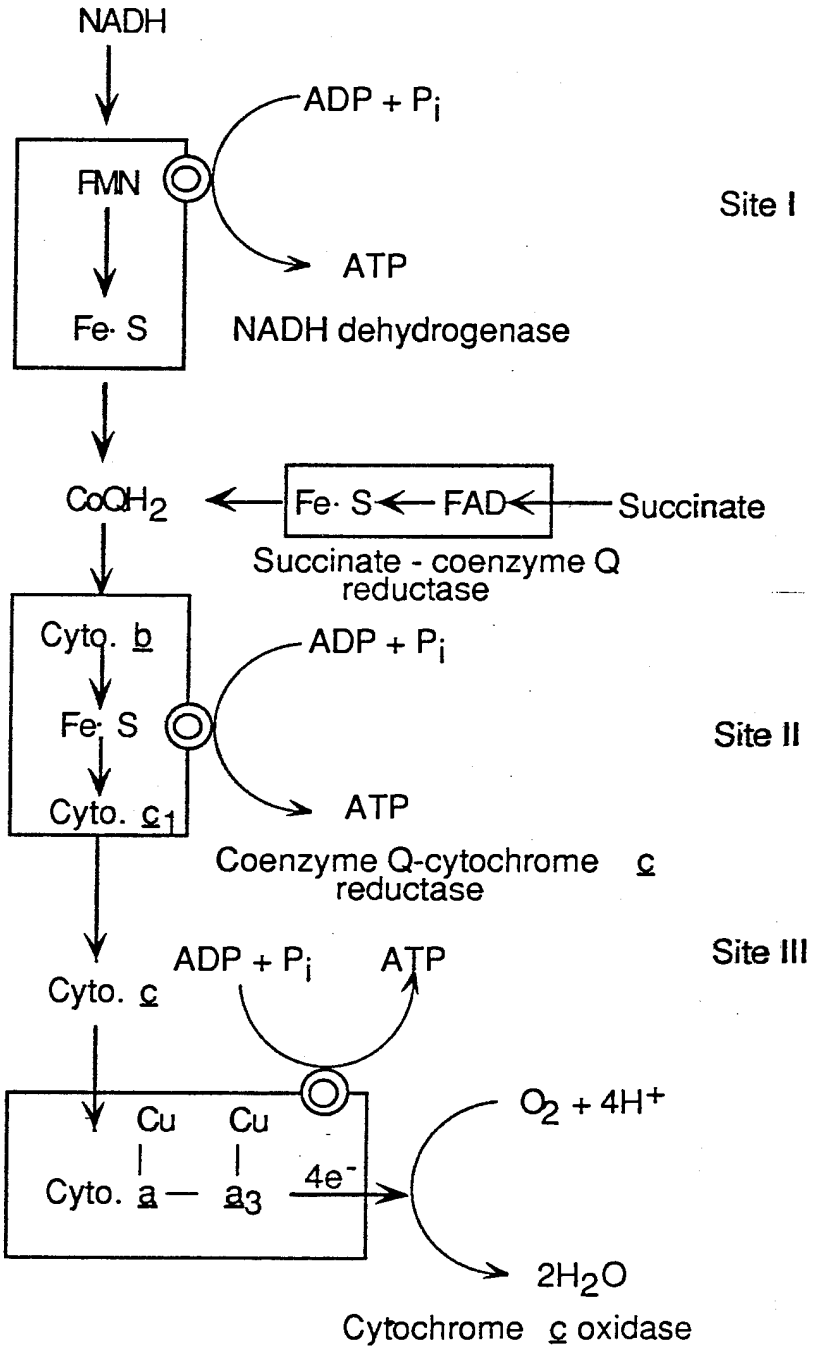

Figure 1: Schematic of mitochondrial respiratory chain showing sequence of electron transport and the three sites of oxidative phosphorylation. (Modified from Piantadosi 1987 p. 29).

Cytochrome a,a_3, located in the inner mitochondrial membrane, catalyzes electron transfer from cytochrome c to O_2. The enzyme complex contains two hemes, two coppers, and multiple (12 or 13) protein subunits. The a and a_3 refer to its two hemes, each heme being associated with one copper atom. The basic structure containing $Fe_aCu_aFe_{a_3}Cu_{a_3}$ is a monomer, where "a" refers to the Fe and Cu associated with $haem_a$ and "a_3" refers to the Fe and Cu associated with $haem_{a_3}$. All metal ions appear to reside in the first three protein subunits. There exists an apparent stoichiometry of 5Cu/4Fe/2Zn/2Mg which supports a dimer of 2Cu/2Fe/Zn/Mg with one additional shared copper (Cu_X). Structural and functional roles of Cu_X, Zn, and Mg remain unclear (Yewey and Caughey 1988). $Haem_a$ and its associated copper, Cu_a, are magnetically isolated indicating they are > 12 A apart, whereas $haem_{a_3}$ and its copper ion Cu_{a_3} are close to one another and act together to bind dioxygen and reduce it to water, stabilizing and retaining in bound form all the intermediates on the pathway (Greenwood et al.1988). $Haem_a$ and Cu_a are thought to act as the electron entry pole of the enzyme and to provide electrons to the binuclear center. Electron entry into $haem_a$ and Cu_a is accompanied by major conformational changes which seem to play a regulatory role on the ligand-binding properties of the binuclear center and electron flux through the enzyme. Although electrons are known to enter the enzyme specifically via the haem of

cytochrome a, the further electron transfer sequence is not known in detail. The sequence $haem_a \rightarrow Cu_a \rightarrow Cu_{a_3} \rightarrow haem_{a_3}$ is often quoted. O_2 binds to the sixth (axial) position of the Fe in $haem_{a_3}$. Fe_{a_3} also binds CO, in the ferrous state and HCN, HN_3, and H_2S in the ferric state. O_2 may bind also to Cu_{a_3} as some recent data suggest (Wikstrom 1988).

Besides being electronmotive, transfering electrons to oxygen, the functioning metals of cytochrome c oxidase are protonmotive, moving hydrogen ions outside the mitochondria to form an electochemical H^+ gradient which transfers energy the final ATP synthesis site in aerobic respiration (Mitchell 1988). A property of cytochrome a,a_3 which allows optical monitoring is that it undergoes marked changes in its spectra as the redox state changes. The redox states of the haems may be monitored by spectroscopic methods at ultraviolet and visible wavelengths, while the coppers have detectable spectra in the near infrared region.

Measurement

There are a variety of methods of determining tissue oxygenation but many are not suitable for human studies. The Fick equation $Vo_2 = Q(Ca_{O_2} - Cv_{O_2})$ assesses oxygen delivery as a function of arteriovenous (A/V) oxygen difference, but A/V difference is a systemic measure and may not be representative of cerebral oxygenation. Even if blood samples were taken from the jugular vein, for example,

superficial facial blood flow and possible shunting would be included in the calculated oxygen level (Piantadosi 1989b). P_{O_2} microelectrodes have been used to measure brain tissue oxygen levels but this is an invasive determination (Torbati 1987). Ear oximetry determines hemoglobin oxygen saturation level, but even if it was representative of brain oxygenation, no information about O_2 utilization is obtained. Magnetic resonance spectroscopy and positron emission tomography (PET) have been used to monitor intracellular oxidative metabolism but are not practical methods for use in a hyperbaric chamber (Giannini et al. 1982).

Non-invasive optical methods have been developed which can be used to assess cerebral and muscular oxygenation by detecting relative changes in the amount of deoxygenated hemoglobin (Hb), oxygenated hemoglobin (HbO_2), and oxidized cytochrome a,a_3 by measuring changes in absorption at specific near infrared wavelengths (Jobsis 1977). An instrument developed to perform near infrared spectroscopy (NIRS) was modified for a hyperbaric chamber and used in these experiments to monitor cerebral oxygenation.

Near Infrared Spectroscopy

Definition and History

In 1925 David Keilin discovered the significance of work done by McMunn in the mid 1880's, who reported on the

spectrum of tissue pigments he called histohematins,(Keilin 1925). Keilin renamed these pigments cytochromes a, b, and c. Warburg named cytochrome oxidase, completing the cytochrome chain's description (Warburg 1949). Optical measurements in intact tissues were pioneered by Britton Chance (Chance 1954). He developed differential spectroscopy to measure changes in visible light absorption by intramitochondrial cytochromes, hemoglobin, myoglobin, and flourometric techniques to measure reduced pyridine nucleotides, all of which are of interest in determining oxygenation levels. Absorption of light by hemoglobin and myoglobin are well known and much of the useful information on the chemical transport mechanism for oxygen has been obtained using spectroscopic techniques. Chance pioneered investigation of the oxygen utilization end of oxygen delivery by taking advantage of the optical properties of enzymes in the mitochondrial respiratory chain (Chance and Williams 1956). Optical studies of NADH and the cytochromes of the respiratory chain are useful in assessing oxidative metabolism. Monitoring of electron transport is useful since it is the end point of most oxygen delivery and is the primary source of free energy conserved by the cell in the form of ATP. Each of the cytochromes has a characteristic absorption spectrum that varies with redox state. The redox states depend on the rate of oxidative metabolism which is determined by availability of oxygen,

reducing equivalents, ADP and P_i for phosphorylation (Balaban 1990).

The differential spectroscopic technique developed by Chance uses the difference in absorption of light in tissue at two nearby wavelengths. The absorption of the reference wavelength is subtracted from the absorption of the sample wavelength. The wavelengths are chosen such that changes in absorption of the sample wavelength correlate to changes in the amount of the species of interest present in the illuminated area. Shifts in the redox state of cytochromes and the amount of hemoglobin, oxygenated and deoxygenated, are reflected by changes in the intensity of light absorbed. Changes in intensity due to light scattering are compensated for by the reference wavelength. Initial *in vitro* work used UV and visible light wavelengths (400 - 700 nm) (Chance 1954). This provided valuable insight into the states of mitochondria and the mechanism for aerobic respiration. However, when measurements are made in tissue, UV and visible light are distorted by complex biophysical obstacles.

When photons encounter tissue, their transmission depends on a combination of reflectance, absorption, and scattering. Reflectance is mainly a function of the angle of incidence. The amount of scattering is inversely proportional to wavelength. Absorbance occurs at specific wavelengths determined by the molecular properties of the materials in the light path (Slayter 1976). Hb is an intense absorber

and scatterer of light but changes in the amounts of hemoglobin and oxygenated hemoglobin can be compensated at visible wavelengths. Spectral interference from other cytochromes also complicate optical measurement in the 400 - 700 nm range. Transmission of light favors longer wavelengths, i.e. near infrared light. Water, which comprises 80% of biological tissue, absorbs all photons with wavelengths over 1300 nm for path lengths over 2 to 3 millimeters. Below 700 nm increased scattering prevents transmission over longer path lengths. Thus near-infrared light, 700 to 1300 nm, is the most effectively transmitted light in measurable amounts over relatively long distances in biological material. The propagation of light or photon migration in brain tissue is highly efficient (20% loss/cm) and relatively independent of wavelength from 630 - 800 nm (<10% change with even smaller changes to 1000 nm). (Jobsis 1977).

Only a few biological chromophores absorb light in the NIR region. These are, notably, haem and Cu ions present in the porphyrins of haem proteins and enzymes. Both forms of hemoglobin, Hb and HbO_2, and the oxidized form of cytochrome a,a_3 have weak but detectable absorption bands in the NIR region. Hb exhibits a weak absorption peak at 760 nm whereas HbO_2 does not. An isosbestic point, where the absorption spectra of two absorbing entities intersect, exists at 805 to

810 nm. Figure 2 shows a representation of the NIR spectra obtained from the brain of a cat in situ.

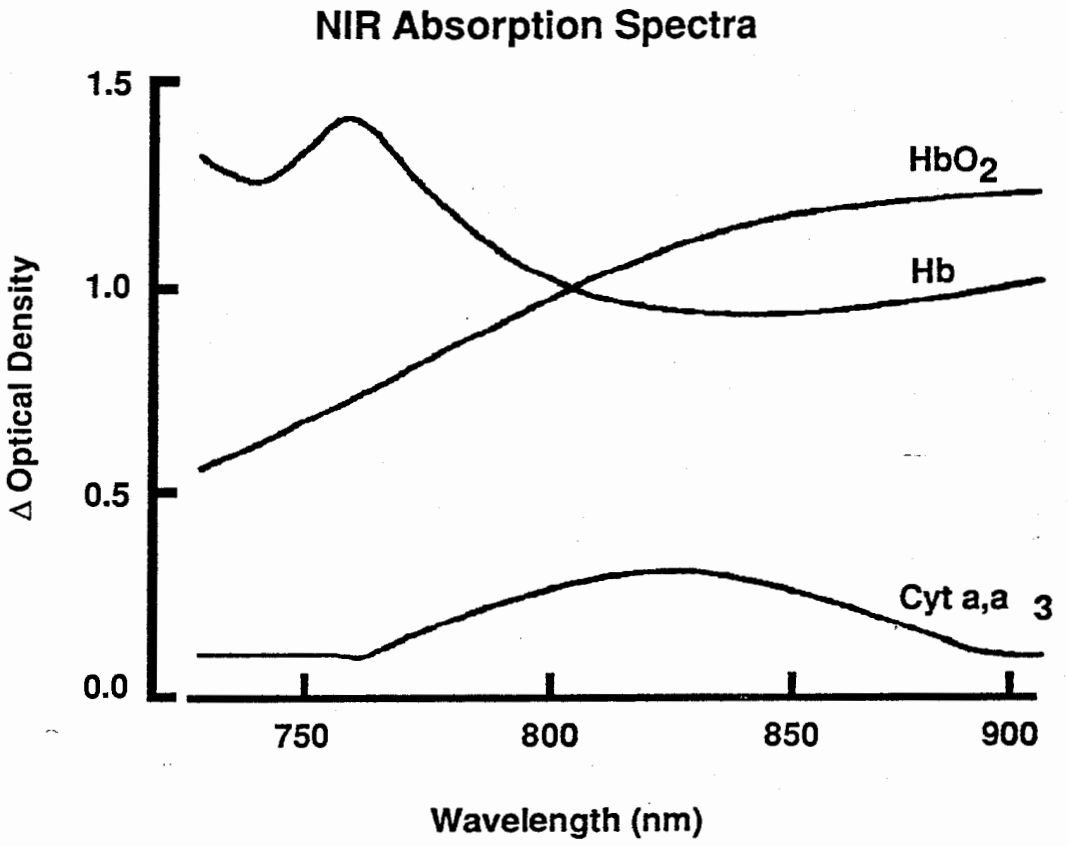

Figure 2: NIR absorption spectra of Hb, HbO_2, and oxidized cytochrome a,a3 (Piantadosi 1989 p. 311).

The absorption band of cytochrome a,a3, when oxidized, is present from 780 to 870 nm with a broad maximum from 820 to 840 nm (Wharton and Tzagoloff 1961). When the enzyme is reduced, which occurs due to a lack of molecular oxygen, this absorption band disappears. The absorption of copper in the

oxidized state at 830 nm peak, may be due to two absorption spectra which have been deconvoluted by Lorentzian analysis, one with a peak at 820 nm and the other with a peak at 870 nm. Since the smaller 870 nm band is of uncertain origin, it is subtracted from the larger band in most biological applications (Jobsis et al. 1988).

In 1977 Jobsis demonstrated the ability of NIR light to be transmitted through skin and bone thus making possible non-invasive monitoring of the oxygenation state of hemoglobin and the redox state of cytochrome oxidase. The contributions of the skin and skin plus bone in small animals was less than 5% of the total signal strength (Piantadosi, Hemstreet, and Jobsis 1986). This has also been confirmed in human skin but not yet for bone (Hampson and Piantadosi 1988). When monitoring over the cranium of larger animals, bone blood flow may be significant. The NIR copper signal from skin and bone is weak because these tissues contain low concentrations of cytochrome oxidase.

Because the spectra overlap, the contributions of Hb, HbO_2, and oxidized cytochrome a,a_3 must be determined using at least three wavelengths to deconvolute the spectra. In some cases four wavelengths can be used to provide more accurate descriptions of NIR absorption. The dual wavelength spectroscopic method, which was later improved upon by using three wavelengths, corrected for equal scattering and absorption at those wavelengths but did not

correct for changes by other absorbers that contribute unequally. Therefore, it was necessary to do animal experiments to isolate the species of interest and determine their relative contributions to the absorption at each wavelength.

Cats were exposed to hyperbaric oxygen to obtain the absorption spectra of fully oxygenated hemoglobin + totally oxidized cytochrome a,a_3. The cats were then perfused with Flourocarbon FC43 which delivers oxygen and removes carbon dioxide while maintaining cellular function without the aid of hemoglobin. Absorbance spectra in the NIR region obtained at this point were therefore due totally to cytochrome a,a_3 oxidized. Initiation of hypoxia led to reduced cytochrome a,a_3 absorption signals. The fully oxidized spectra minus the cytochrome c oxidase spectra resulted in the HbO_2 contribution. The blood that was originally exchanged for FC43 was washed with 10mM $Na_2S_2O_4$ to deoxygenate the hemoglobin. The blood was then exchange perfused back into the cat to obtain the Hb spectrum. Similar experiments were performed on intact rat brains (Piantadosi 1989b).

Algorithms are derived from NIR spectra obtained from the animal experiments using the following procedure. Three linear equation are written in the following form:

$$\Delta OD_1 = a(\Delta OD_{M1}) + b(\Delta OD_{M2}) + c(\Delta OD_{M3})$$
$$\Delta OD_2 = d(\Delta OD_{M1}) + e(\Delta OD_{M2}) + f(\Delta OD_{M3})$$
$$\Delta OD_3 = g(\Delta OD_{M1}) + h(\Delta OD_{M2}) + i(\Delta OD_{M3})$$

where: ΔOD_{Mn} = the change in optical density for the spectra of molecules, n = 1-3
a-i = fractional absorption values
ΔOD_n = the change in optical density for three wavelengths (n = 1-3).

These linear expressions are solved simultaneously by matrix inversion yielding the following equations:

$$\Delta OD\ M1 = a^{-1}(\Delta OD_1) + b^{-1}(\Delta OD_2) + c^{-1}(\Delta OD_3)$$
$$\Delta OD\ M2 = a^{-1}(\Delta OD_1) + b^{-1}(\Delta OD_2) + c^{-1}(\Delta OD_3)$$
$$\Delta OD\ M3 = a^{-1}(\Delta OD_1) + b^{-1}(\Delta OD_2) + c^{-1}(\Delta OD_3)$$

where:

$\Delta OD\ Mn$ = concentration change of molecules measured, n = 1-3
a^{-1}-c^{-1} = the weighting coefficients
ΔOD_n = the change in optical density at three wavelengths (n = 1-3).

Optical density or absorbance is given by the Beer-Lambert relationship:

$$OD = \log I_O/I = \varepsilon cl$$

where:

I = Intensity of transmitted light
I_O = Intensity of incident light
ε = Absorption coefficient
c = Concentration of absorbant species
l = Path length

The algorithms relate changes in the relative concentrations of tHb, $tHbO_2$, and oxicized cytochrome a,a_3 to each other according to the total absorption change at each of three wavelengths. Instead of true absorbance or optical density, measurements are given as variations in density or

v.d. because path length is not known. The v.d. are linearly related to changes in concentration, and one v.d. unit has been defined as a 10-fold change in the amount of the appropriate absorber as computed by the algorithm (Jobsis 1985).

In order to obtain true concentrations, the change in optical density must be divided by path length. Accurate path length determinations for reflected light have not yet been determined but many techniques to measure path length are currently being investigated. Attempts have been made to calculate path length by generating point spread functions using a Monte Carlo technique for light in tissue for a generalized range of characteristics (Van Der Zee and Delpy 1988), by using random walk computation based on 100 micron discrete paths (Bonner et al. 1987), by comparing light travel time in water with light travel time in the brain (assuming the brain is 80% water) (Wray et al. 1987), and by time-resolved measurements (Chance et al. 1988).

Ventilatory Response

History

The control of respiration is such a complex system that it is useful to study it in terms of responses to stimuli. CO_2 is the most commonly used chemical stimulus and ventilation the most commonly measured response utilized to investigate various mechanisms involved in respiratory

control (Rebuck and Slusky 1981). Measurement of ventilatory response to increased inspired CO_2 has been used as a tool to study human respiration since the turn of the century when Haldane and Priestley quantified the effect of CO_2 on ventilation. Ventilatory response to CO_2 has been quantified in two ways, the steady state and rebreathing techniques. The steady state method consisted of having subjects breathe different gas mixtures (at least 3) containing different amounts of carbon dioxide for 5 - 15 minutes and measuring ventilation during each time period. This method was tedious, time consuming, and unpleasant.

The rebreathing method has the subject breathe from a spirometer so that the P_{CO_2} in the system rises secondary to the subject's own CO_2 elimination. Once the P_{CO_2} in the system closely matches the brain Pt_{CO_2}, the ventilation/ P_{CO_2} relationship is linear. The slope of ventilation vs. P_{CO_2} is an indicator of an individual's response to a CO_2 stimulus (Rebuck and Slusky 1981).

Increased inspired oxygen partial pressures lead to decreased ventilatory response to CO_2 and possibly to carbon dioxide retention (Lambertsen 1978). Individuals that retain CO_2 may be more susceptible to CNS oxygen toxicity (Lanphier 1975). CO_2 retention may lead to cerebral vasodilation thus increasing cerebral oxygenation and increasing the risk of CNS oxygen toxicity.

3. METHODS

Subjects

This study was approved by the Duke University Institutional Review Board. The study was supported by the Office of Naval Research. Eleven normal healthy adult volunteers (9 males, 2 females), ages 23 - 48, gave written informed consent to participate in all studies. Subjects were compensated $100 per study. Each subject was familiarized with the experimental environment and procedures before testing. All subjects had normal chest x-rays and diving physicals within one year of testing as per protocol for exposure to hyperbaric pressure. Subjects were advised of the potential symptoms of oxygen toxicity prior to the study and told that they could stop at any point during the experiment if they became too uncomfortable. A breakdown of the number and type of experiments performed as well as subject characteristics are included in Table 1.

Table 1

Subject Information

SUBJECT	SEX	AGE	HEIGHT in.	WEIGHT lb.	NO. OF EXPERIMENTS (W/HYPOXIA)
AE	M	23	70	165	4 (1)
AH	F	35	67	115	1 (0)
CM	M	32	74	215	8 (3)
CP	M	40	69	185	2 (1)
DS	M	34	73	205	3 (1)
MN	M	28	70	185	2 (2)
OD	M	43	71	210	5 (3)
RV	M	48	70	160	3 (3)
SH	F	33	64	155	1 (0)
TA	M	32	65	150	4 (2)
TS	M	43	70	175	1 (1)

Experimental Protocol

Cerebral oxygenation and ventilatory response to CO_2 were monitored simultaneously during either progressive normocapnic hypoxia or progressive hypercapnia at one of three oxygen pressures. Table 2 summarizes the experimental protocol. The progressive hypoxia exposure was not performed in 17 of 34 experiments .

Table 2

Experimental Protocol

Normocapnic Normoxia	5 min
Progressive Normocapnic Hypoxia to 70% Sa_{O_2}	9 min
Normocapnic Normoxia	15 min
Normoxia / Progressive Hypercapnia to PET_{CO_2} = 60 torr	5 min
Normocapnic Normoxia at 77 fsw	15 min
Hyperoxia (2.80 ATA) / Progressive Hypercapnia to PET_{CO_2} = 60 torr	5 min
Normocapnic Normoxia at 35 fsw	15 min
Hyperoxia (1.75 ATA) / Progressive Hypercapnia to PET_{CO_2} = 60 torr	9 min

Subjects were advised of possible symptoms and instructed to give a report of any type of symptom immediately after the mouthpiece was removed. A member of the Hyperbaric Center staff accompanied subjects in the chamber throughout the entire experiment. Subjects were seated comfortably in a semi-reclining posture. Two optical fiber bundles, optrodes, (transmitting and receiving) were coupled to a head band. The head band was positioned to ensure that the optical fiber bundles were directed away from the frontal sinus. The band

was tightened to blanch the skin of blood flow beneath the optrodes. The optical fiber bundles were kept in the same place on the subject's forehead throughout the entire experiment in order to allow comparison of the signals for each condition. Exact placement of the optrodes was determined by adjusting the headband until a strong stable signal was acquired. During testing, the subject wore a nose clip and breathed through a SCUBA mouthpiece connected to the closed-circuit rebreathing apparatus.

The experiment started by having the subject exhale twice into the breathing circuit, attach the nose clips and start normal respiration through the mouthpiece. The breathing media was maintained at 0.2090 ± .01 ATA PI_{O_2}. Initially, all exhaled flow passed through a canister containing sodasorb, which chemically binds CO_2 and therefore eliminates CO_2 from the inspired gas. Normocapnic normoxia was maintained for 5 minutes and comprised the control for each experiment.

For hypoxic exposures, oxygen was not added to the breathing circuit. Rebreathing resulted in a linear decrease in PI_{O_2} to 0.05 ATA and consequent reduction in arterial oxygen saturation to 70% saturation as recorded by a finger pulse oximeter. PET_{CO_2} was maintained at the value achieved after the five minute control period (± 2 torr) by manually adjusting the exhaled flow through the sodasorb canister and a canister by-pass. When the 70% saturation end point was achieved, oxygen was added to the rebreathing circuit and the

PI_{O_2} was maintained at 0.2090 ± .01 ATA for 2 minutes. The subject then came off of the mouthpiece and breathed chamber air for 15 minutes before beginning the next part of the protocol.

During hypercapnic portions of the protocol, flow to the sodasorb canister was blocked and rebreathing resulted in an increase in PET_{CO_2} to 60 torr. PI_{O_2} was maintained at the desired level 0.2090 ± .01 ATA at the surface. Once the 60 torr PET_{CO_2} end point was reached, exhaled flow was diverted through the sodasorb canister until the initial PET_{CO_2} was maintained for 2 minutes. After removing the mouthpiece and breathing chamber atmosphere, the chamber was pressurized to 77 fsw, the system was calibrated and the breathing circuit was flushed with oxygen until it contained 85% O_2. This took 15 minutes after which a hypercapnic exposure was conducted with a PI_{O_2} of 2.80 ± 0.005 ATA.

After completion of the hypercapnic exposure at 77 fsw, the chamber was decompressed to 35 fsw. The system was recalibrated and flushed with oxygen. Another hypercapnic exposure was conducted at a PI_{O_2} of 1.75 ± 0.005 ATA. Nitrogen absorbed by tissues during air breathing at 77 fsw is "washed out" during oxygen breathing. Nitrogen washout increased as a result of decompression to 35 fsw. Excess volume introduced into the breathing circuit and was eliminated by extracting gas from the system with the calibrated syringe through a three-way valve.

Following the hypercapnic exposure at 35 fsw, the chamber was decompressed to 30 fsw. Both subject and tender breathed oxygen to fulfill decompression commitments acquired during the dive.

Equipment

Computer

Data were recorded on a DEC MINC 11/03 computer on a breath by breath basis, triggered at end exhalation. The following variables were sampled at 50 Hz by a 12 bit A/D board installed in the MINC 11/03: inspiratory temperature (°C), expiratory temperature (°C), spirometer temperature (°C), inspiratory oxygen partial pressure (ATA), expiratory oxygen partial pressure (ATA), volume (L), peak-peak mouthpiece pressure (cm H_2O), oxygen saturation (% Sa_{O_2}), and heart rate. Input voltage range was +5 to -5 volts, allowing 2.44 millivolt resolution. The controlling software, VRPRO.FOR, was written in Fortran IV. Subroutines were developed for system calibration, data aquisition, display, and storage, and oxygen control.

Spirometer and Breathing Loop

The closed-circuit breathing apparatus depicted in Figure 3 is based upon a 9 liter rolling seal spirometer (Sensormedics model 922).

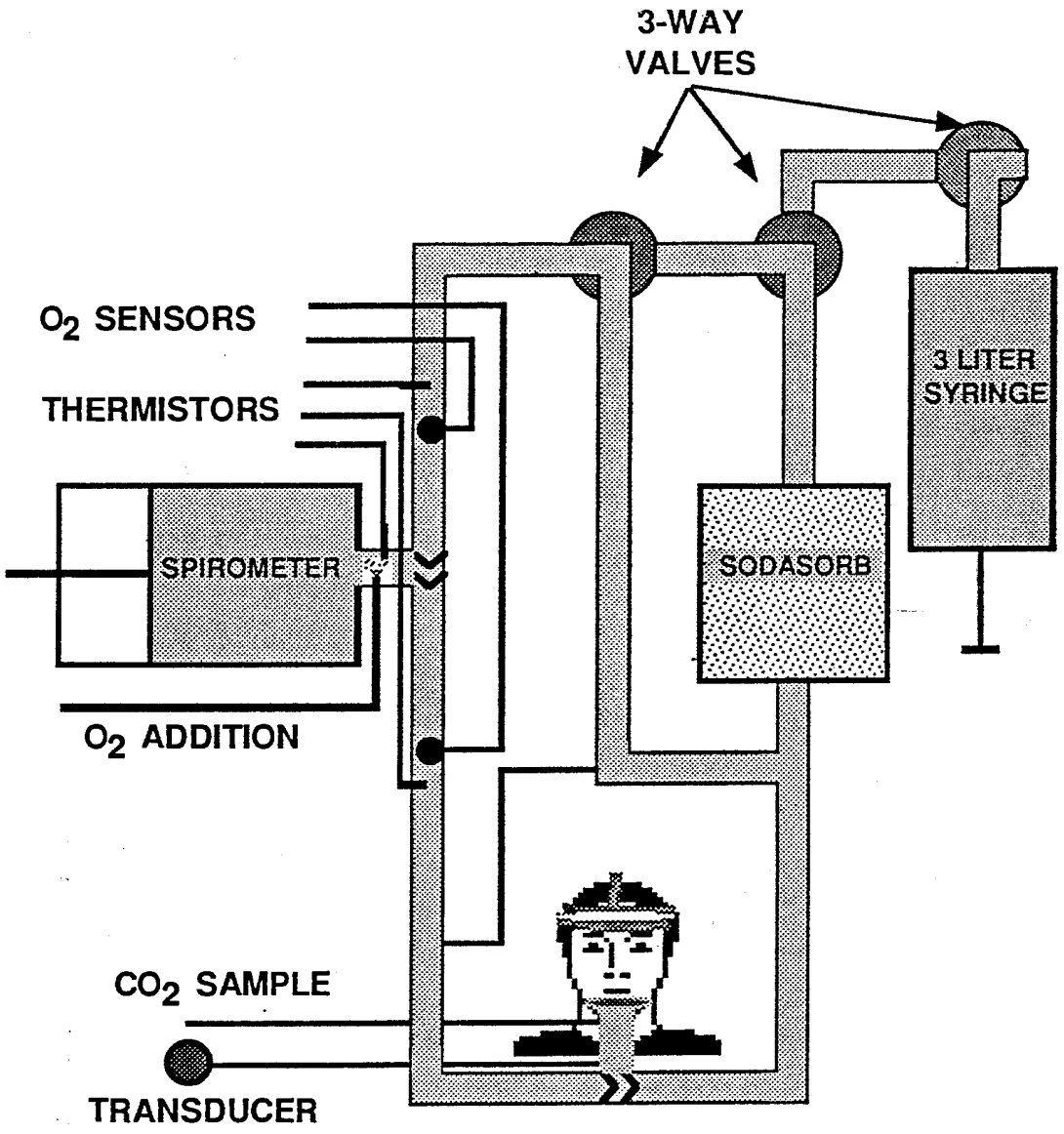

Figure 3: Closed-circuit breathing apparatus used to achieve oxygen and carbon dioxide pressures required for experimental conditions.

A potentiometer supplied with + 5 to -5 volts was attached to the piston of the spirometer and provided voltage output to the computer. The spirometer is calibrated with a 3 liter

syringe (Collins model M-20). A temperature probe (YSI model 710 thermistor) was inserted into the entrance of the spirometer to provide temperature correction for volume measurements.

The model 710 thermistor, time constant = 0.3 sec, was interfaced with the computer through an amplification circuit (Figure 4) and was calibrated from 17 - 37 °C. Tidal volume (V_T) was corrected to BTPS as follows:

$$V_T = (V_{max} - V_{min}) * T_{Cor} * WV_{Cor}$$

where:

V_{max} = Maximum volume during breath
V_{min} = Minimum volume during breath
T_{Cor} = Temperature correction = $310.0/(273.0 + T_S)$
T_S = Spirometer temperature

$$WV_{Cor} = (P_{ATA} - P_V) / (P_{ATA} - P_{V_{BT}})$$

where :

P_{ATA} = Barometric pressure in ATA
P_V = Vapor pressure in ATA.
$P_{V_{BT}}$ = Vapor pressure in ATA at body temperature

Vapor pressure was determined using the following third order polynomial :

$$P_V = (2.47488 + (0.65949*T_S) - (0.00712*T_S^2) + (0.00059*T_S^3)) / 760.0$$

This expression was obtained by doing polynomial regression of tabular data giving vapor pressures for temperatures from 20 °C to 37 °C.

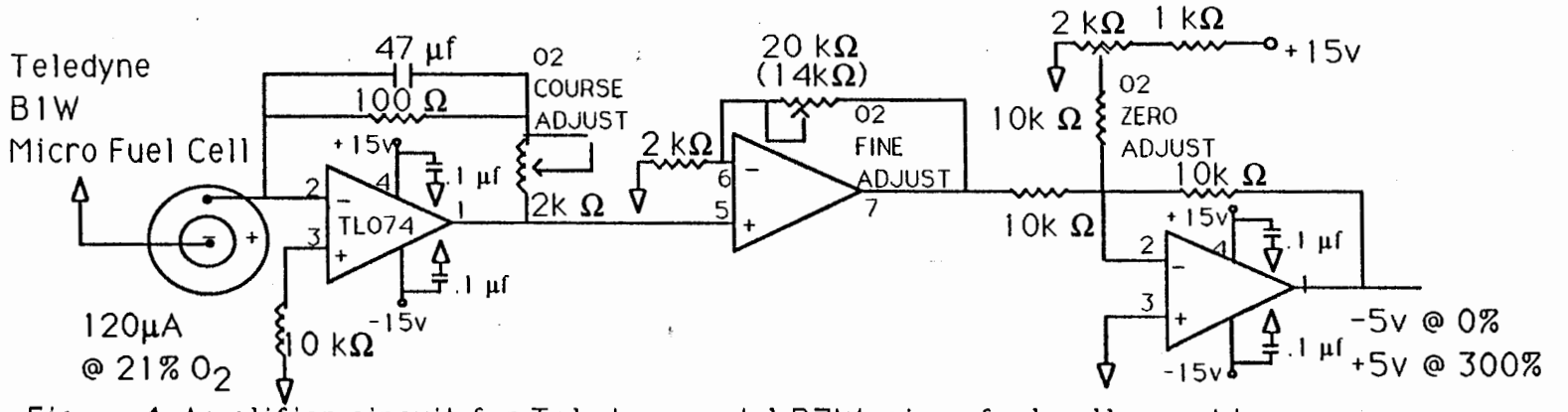

Figure 4: Amplifier circuit for Teledyne model B7W micro fuel cells used to determine oxygen partial pressures within the closed-circuit breathing apparatus.

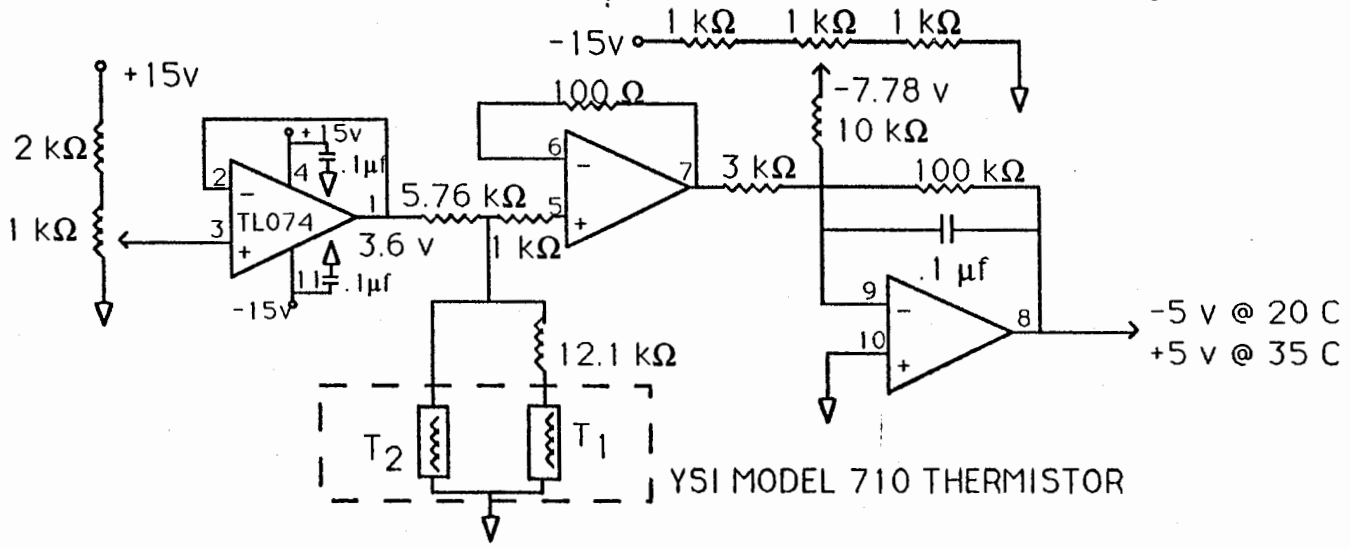

Figure 5: Amplifier circuit for YSI model 710 thermistor used to provide temperature compensation for volume and oxygen partial pressure determinations.

Maximum and minimum volumes were determined using a peak detection software routine to obtain V_T. Respiratory rate was determined by measuring the time between maximum volumes or end-exhalations. Respiratory minute volume (RMV) was calculated as:

$$RMV = V_T \times \text{respiratory rate}$$

Flow was directed into and out of the spirometer via a T-shaped two way valve (Hans Rudolf model 2700). This valve allowed for better separation of inspired and expired oxygen pressure determinations and provided an entry point for the spirometer temperature probe and an oxygen addition input. Another valve (Hans Rudolf model 2700) served as the mouthpiece valve. A silicon rubber SCUBA mouthpiece was attached to a microbial filter (Pall P30S) which was then attached to the valve. These valves were chosen for their low dead space, 90 ml, large bore, $1\frac{1}{8}$ in., and low resistance (0.8 cm H_2O lpm^{-1}). Breathing resistance, which increases with density of the breathing gas, was minimized by ensuring that all portions of the breathing loop had an inner diameter (ID) of at least $1\frac{1}{8}$ in. $1\frac{3}{8}$ in. ID smooth bore tubing (Hans Rudolf model 9039) was used to connect the components of the breathing circuit.

Oxygen Pressure Control

Micro fuel cells (Teledyne Analytical model B1W) were used as oxygen sensors to monitor the inspired and expired

partial pressures of oxygen. The fuel cells had a response time of 7 seconds. Each was mounted into a $1\frac{1}{4}$ in. copper "T" on opposite sides of the two-way valve at the entrance to the spirometer. The fuel cell surface was exposed to the gas flow but did not obstruct the breathing loop. Temperature compensation was provided by YSI model 710 thermistors which penetrated the copper tees in close proximity to each oxygen sensor. The current output from each micro fuel cell was input into current to voltage conversion and amplification circuitry (Figure 5). The oxygen sensors were calibrated at each depth using primary standard calibration gases (National Specialty Gases). Nitrogen was used as the "zero" gas. The "high" calibration gases were 20.90% O_2 at the surface, and 96.00 % O_2 at 35 and 77 fsw. Temperature compensation was accomplished in software using output from YSI model 710 thermistors which penetrated the copper tees in close proximity to each oxygen sensor. Software temperature compensation was achieved through the use of a difference function derived from data obtained during testing of micro fuel cell output as a function of temperature. The following correction was applied on a breath by breath basis to oxygen sensor output:

$$O_{2cor} = O_{2out} - (0.0074062*(T_{O_2} - T_{Cal}))$$

where :

O_{2cor} = temperature compensated oxygen output in ATA
O_{2out} = uncompensated oxygen output in ATA
T_{O_2} = temperature at the oxygen sensor during the last breath
T_{Cal} = temperature at the oxygen sensor during calibration

Oxygen addition was directed by a computer controlled solenoid valve connected to a regulator (National Welders model HPTD) on a G-cylinder of 99.99% USP O_2. Oxygen flowed from the solenoid through a 60 micron filter and a 0.0135 in. ID orifice designed to achieve a critical flow of 6 liters per minute. A constant mass rate of gas flow, critical flow, is achieved when back pressure is less than half of the supply pressure at stagnation conditions for a given orifice size (Harter 1967). When the inspired oxygen partial pressure dropped below the user defined set point by an input amount, the solenoid was fired for 1.0 second, adding approximately 100 ml of O_2. Fluctuations about the set point were minimized by adjusting the regulator setting during the expeiment. The regulator was initially set at 30 psi at the surface, 60 psi at 35 fsw and 90 psi at 77 fsw. Comparisons to the set point were done every other breath to allow for gas mixing delays. Computer control was achieved using an output from a digital I/O board within the MINC 11/03

computer. To fire the solenoid, the least significant bit of the 8 bit I/O port was set high (+4.5 volts) and sent to the input of a 12 V solenoid driver.

Carbon Dioxide Control

A gas sample was drawn from the mouthpiece valve by the pump in a Beckman LB2 Carbon Dioxide Analyzer, used to obtain PET_{CO_2} determinations. The gas was sampled at 200 ml/min and returned to the rebreathing circuit at a point in the exhalation side of the spirometer to avoid gas loss due to sampling. The analyzer's response time is 0.1 sec. The CO_2 analyzer was calibrated at each depth using primary standard calibration gases, 8.00% CO_2 at the surface, 4.00% CO_2 at 35 fsw, and 2.52% CO_2 at 77 fsw. The output from the analyzer was input into the computer and converted from a surface equivalent percentage to units of torr (8.00% = 60.80 torr at 1 ATA) . The CO_2 analyzer was modified to allow a nitrogen purge to be installed proximal to the analzyer's pumps, power supply and infrared source to eliminate the chance of a spark or heat build-up, fire hazards in a hyperbaric chamber. Gas samples were taken from within the analyzer during the compression of the chamber to ensure that less than 4% oxygen remained within the analyzer's electronics compartments. This limited the rate of compression of the chamber to 15 ft/min.

A peak detection software routine was employed to determine the maximum PET_{CO_2} per breath. CO_2 levels were controlled manually by the tender by manipulating two three-way "Y"-shaped stopcocks (Hans Rudolf 4000A). The CO_2 level could be maintained to within 2 torr of a setpoint. Use of the stopcocks allowed exhaled flow to go through the sodasorb canister (Collins model 21377), by-pass it, or go through both. The sodasorb chemically binds CO_2 via the following reaction (W.R. Grace and Co. 1962):

i.) $CO_2 + H_2O \leftrightarrow H_2CO_3$

ii.) $2H_2CO_3 + 2Na^+ + 2OH^- + 2K + 2OH^- \rightarrow 2Na^+ + CO_3^= + 4H_2O$

iii.) $Ca(OH)_2 + H_2O \leftrightarrow Ca^{++} + 2OH^- + H_2O$

iv.) $2Ca^{++} + 4OH^- + 2Na^+ + CO3^= + 2K^+ + CO_3 \leftrightarrow$
$2CaCO_3 + 2Na^+ + 2OH^- + 2K^+ + 2OH^-$

The reaction is exothermic, 13.5 kcal/gram molecular wt. CO_2, thus heating the exhaled gas. The brief durations (5-9 min.) of each part of the experiment prevented large increases in temperature due to the exothermic reaction. Therefore, an in-line heat exchanger was not necessary for this study.

Ancillary Parameters

A fitting was placed in the mouthpiece valve to accommodate $\frac{1}{8}$ in. ID tubing connected to a pressure transducer (Validyne Model MP45-871). The pressure transducer was used to record peak-peak mouthpiece pressure,

an indicator of breathing resistance (Middleton and Thalmann 1981). The mouthpiece pressure was calibrated from 0 - 40 cm H_2O pressure using an electronic manometer (Setra Systems Inc. Model 339-1).

% Sa_{O_2} was measured by finger oximetry (Nellcor pulse oximeter model 100) during the hypoxia exposures down to 70%. Heart rate was also monitored using the same device.

NIRS Instrument

The NIRS instrument used in the study uses laser diodes as monochromatic light sources. Laser diodes have narrow bandwidths (1.5 nm) and can be pulsed rapidly in succession for multiwavelength applications. These are p-n junction devices optimized for radiant output. The Ga-Al-As diodes used have high band gaps resulting in wavelengths in the NIR, 775, 810, 870, and 904 nm. Using time-domain multiplexing, the laser diodes are pulsed at a frequency of 1 KHz with a pulse width of 200 ns.

Fiber Optic Bundles

The light is transmitted and recovered through optical fiber bundles (optrodes). Non-coherent optical fiber bundles are used to prevent bias due to spatial illumination differences. Both optrodes were potted into brass penetrators, allowing for their use in the hyperbaric chamber. The optrodes used are 4 meters in length. At their

ends inside the chamber, the optical fibers were potted into aluminum terminators 1 cm in diameter with polished ends. These terminators allowed the optrodes to be securely fastened to a head band which positions the centers of the fiber bundle terminators 3.5 cm apart. The transmitting and receiving optrodes form an angle of approximately 30 degrees with each other.

Signals

Photons enter the tissue after passing through the skin and bone, travel a distance via multiple reflection and scattering, and emanate at different distances from the entry point. A fraction of these photons is captured by the receiving bundle and conveyed to the detector. A minimum of 2 cm separation of input and pick-up fiber bundles is necessary to prevent back scattered incident light from entering the signal detector. Some optical fibers in the transmitting optrode are designated to carry backscattered incident light to a separate detector as a reference signal since absorbance is measured as a ratio of incident light intensity to collected light intensity. The transmitting fiber optic bundle is therefore split into five legs at the instrument, one carrying backscattered light to the reference photodiode, and the four others carrying light from the four laser diodes to the tissue. The instrument end of the receiving optrode terminates as an aluminum block fitted to

interface with a photomultiplier (Hamamatsu model R936). All connections are made using mechanical fasteners and optical coupling gel (Math Associates Inc.) is placed at all interfaces including the skin/optrode interface.

The photocurrents from the two photodetectors, the reference photodiode and the signal photomultiplier, are integrated, demultiplexed, and fed through a log ratio amplifier. The log signal/reference voltages are then input to a dedicated microprocessor, where algorithms are applied and the metabolic signals are displayed in real time (5 second time constant) on a printer (Epson model FX 100).

Validation

NIRS validation experiments were done using fluorocarbon emulsion, FC-43, exchange transfusion in cats and rats. Statistical analyses indicate an error rate in the determination of cytochrome a,a_3 of approximately 2% in these experiments assuming that changes in cytochrome a,a_3 calculated by the algorithm during the washout are due only to crosstalk from Hb. These experiments verified that algorithms and instrumentation used determine relative changes in the concentrations of Hb, HbO_2, and cytochrome a,a_3 in the illuminated area accurately and independently (Piantadosi, 1989b).

Data

Cerebral Oxygenation

The NIR spectrophotometer provides derived measures of deoxygenated hemoglobin (tHb), oxygenated hemoglobin ($tHbO_2$), the sum of tHb and $tHbO_2$ or blood volume (tBV), and the concentration of oxidized cytochrome a,a_3. tHb, $tHbO_2$, and tBV pertain to small cerebral blood vessels and cytochrome a,a_3 to superficial cortical tissue. These measures are expressed as differences from normoxic, normocapnic, normobaric control values for each subject. Signals were recorded graphically and numerically on the printer. Cerebral oxygenation measurements for no inspired CO_2 data were taken from the printer output after the subject had been breathing a constant PI_{O_2} for 2 minutes. Maximum CO_2 values correspond to measurements taken simultaneously with breathing of gas with a PET_{CO_2} of 60 torr. Figure 6 shows an example of signals recorded by the NIR spectrophotometer during a hypercapnic exposure.

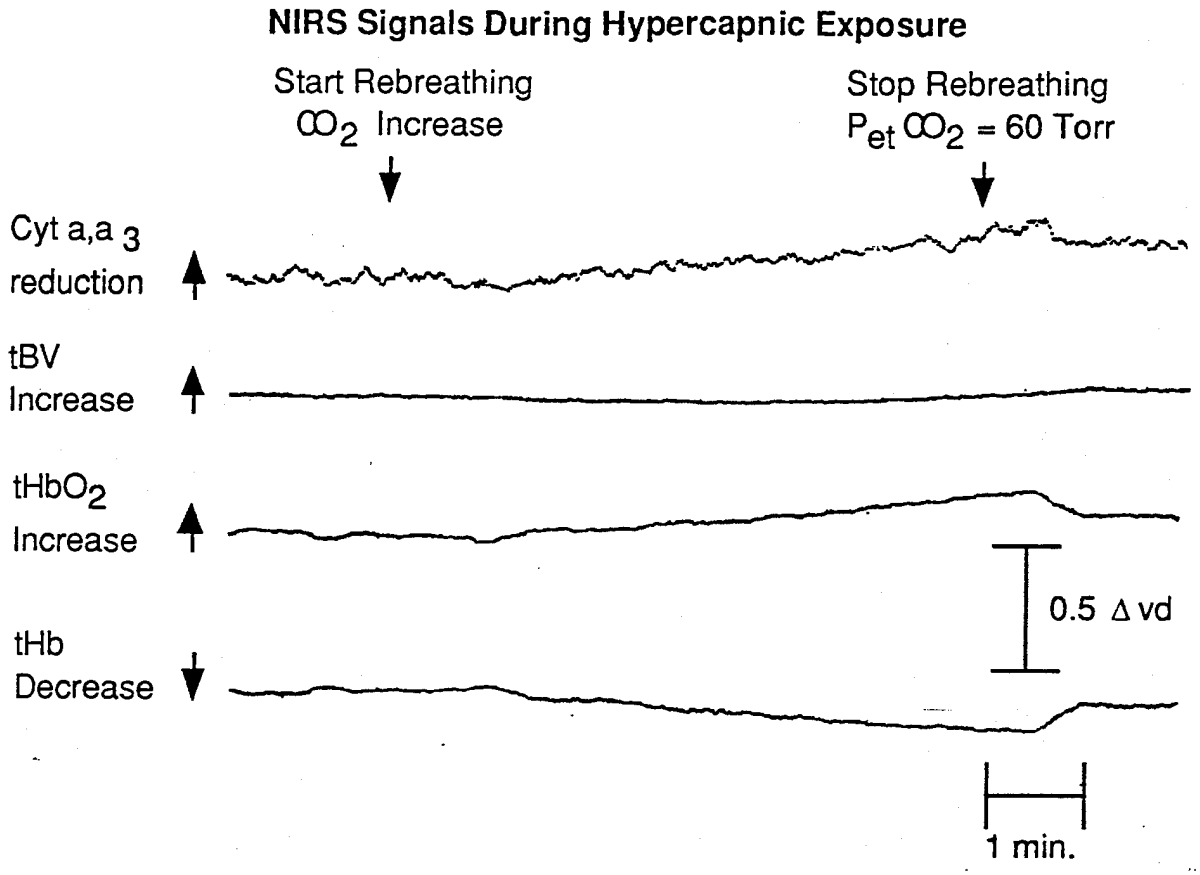

Figure 6: NIRS signals during hypercapnic exposure to 60 torr PET_{CO_2} at PI_{O_2} = 0.21 ATA.

Ventilatory Response

Ventilatory response data is given as the slope of the linear regression of respiratory minute volume (RMV) as a function of PET_{CO_2} from 45 to 60 torr (Figure 7). Data was imported to Cricket Graph ver. 1.3.1 (Cricket Software) on a Macintosh computer, where the data was graphed and linear regression performed.

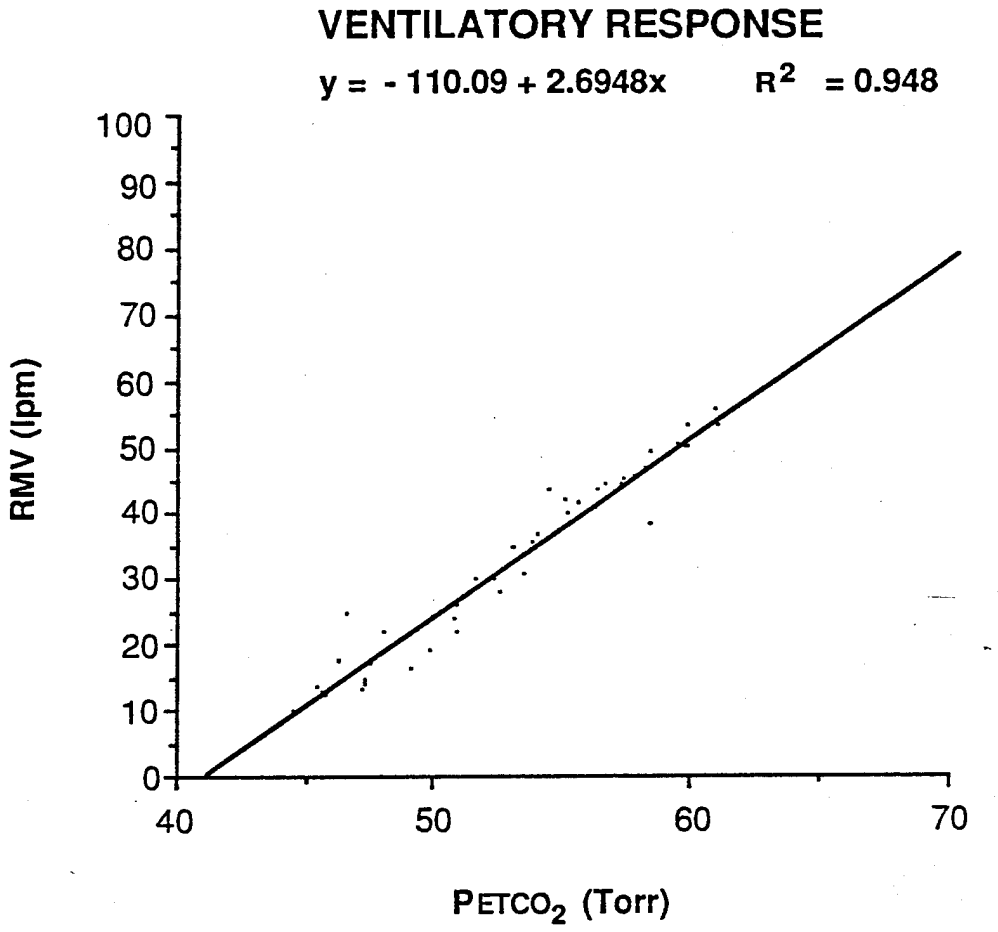

Figure 7: Ventilatory response to CO_2 determination from data recorded during a hypercapnic exposure at 0.21 ATA PI_{O_2}.

Statistics

Cerebral oxygenation data are presented as mean ± standard deviation (SD) for all subjects completing all hypercapnic portions of the protocol (n=30). Hypoxic data were obtained for half of these experiments (n=15). Ventilatory response data are depicted as mean ± SD for all

subjects completing all hypercapnic portions of the experiments (n=32) and for repeated experiments by the same subject (n=2-8). Significant differences were identified by paired and unpaired t tests, the nonparametric Kruskal Wallace test and repeated measures analysis of variance, Fisher PLSD, using Statview II on a Macintosh computer.

4. RESULTS

Symptoms

Definite symptoms (tunnel vision, tinnitus, and extreme anxiety) associated with CNS oxygen toxicity (Butler and Thalmann 1984) were reported within 3 torr of the maximum $P_{ET_{CO_2}}$ at 2.80 ATA $P_{I_{O_2}}$ in 7 of 34 experiments. In one instance, the subject stopped the study by removing the mouthpiece during the hypercapnic exposure at 2.80 ATA $P_{I_{O_2}}$. $P_{ET_{CO_2}}$ was 57 torr at that point. The subject reported extreme anxiety and a feeling of impending unconsciousness. The subject recovered within minutes and the experiment was discontinued. Minor symptoms possibly related to CNS oxygen toxicity (tingling, numbness, narcosis, sweats, and dizziness) were reported under the same conditions in 14 other experiments. Table 3 lists the symptoms reported and their frequency. Multiple symptoms were reported in 15 experiments.

Table 3

Symptom Frequency ([a] Definite symptoms)

SYMPTOM	NUMBER
Sweating	8
Narcosis	7
Numbness	5
Feeling of impending unconsciousness [a]	3
Extreme anxiety [a]	3
Tunnel vision [a]	3
Dizziness	2
Light-headedness	2
Tingling	1
Tinnitus [a]	1
Headache	1

Incidence of symptoms was higher in experiments which included hypoxic exposure (13/17) than in experiments without hypoxia (8/17), but not significantly ($p=0.078$ by Chi-square test without Yates correction). Four out of seven definite symptoms occurred in experiments without hypoxic exposure.

Cerebral Oxygenation

The results of monitoring cerebrocortical oxygenation during hyperoxic CO_2 rebreathing, demonstrate the vasoconstrictive effect of oxygen in the cerebral vasculature, the release of that effect by increased carbon dioxide pressure, intact metabolic regulation of mitochondrial metabolism at high PI_{O_2}, and a reduction in vascular response to CO_2 in individuals displaying symptoms. All cerebral oxygenation data are listed in tabular form in Appendix 1.

Hypoxia

Initially, hypoxic exposures were included in the experimental protocol to verify that modifications done to adapt the NIRS instrumentation for use in the hyperbaric chamber did not effect its measurements. The changes from baseline measured during progressive normocapnic hypoxia to 70% Sa_{O_2} correspond well with data obtained from a similar study (Hampson et al., 1990). Table 4 shows a comparison of data recorded by the spectrophotometer during progressive normocapnic hypoxia to 70% Sa_{O_2} from both studies using different breathing circuits and optics. The values listed below are changes in variation in density from the control condition, breathing a normoxic, normocapnic gas mixture, to the maximum hypoxic condition, Sa_{O_2} = 70 % as determined by finger pulse oximeter. At maximum hypoxia, subjects were

generally breathing 0.05 ATA PI_{O_2} and PET_{CO_2} = 40 torr, normocapnia was maintained. The table shows similar increases in the amount of deoxygenated hemoglobin and blood volume and decreases in oxgyenated hemoglobin and the amount of oxidized cytochrome a,a_3 under the same hypoxic stress. Based on the similarities in the values obtained from the studies, the modifications to the optical fibers had no effect on the spectrophotometer's monitoring capability.

Table 4

Comparison of NIRS Signals from Hypoxic Exposures

Study	Hb	HbO_2	BV	CYT
Previous [a]	+0.27±0.02	-0.19±0.01	+0.08±0.01	-0.15±0.02
Present [b]	+0.25±0.08	-0.22±0.10	+0.03±0.06	-0.12±0.13

[a] Hampson et al., 1990, n = 8
[b] n = 15

Mean Cerebral Oxygenation Changes

Figure 8 presents cerebral oxygenation data as overall group means ± SD for NIR signal changes relative to normoxic control without inspired CO_2 (PI_{O_2} = 0.21 ATA, PET_{CO_2} = 40 torr) for hypoxia (0.05 ATA PI_{O_2}) and maximum CO_2 (PET_{CO_2} = 60 torr) at 0.21, 1.75, and 2.80 ATA PI_{O_2}. Data corresponding to (0.05) ATA PI_{O_2} are the mean ± SD for 15 experiments. Cerebral oxygenation data from normoxic and hyperoxic

exposures are represented as the mean ± SD for 30 experiments. Data from 4 of the 34 experiments conducted were not included due to excessive signal noise or an incomplete experiment. Two of the four included hypoxic exposures. One of the four excluded experiments had a reported definite symptom, one had a reported minor symptom, and two had no symptoms. The order of exposures for all experiments was hypoxia (if administered), hypercapnia at 0.21 ATA O_2, hypercapnia at 2.80 ATA O_2, and hypercapnia at 1.75 ATA O_2.

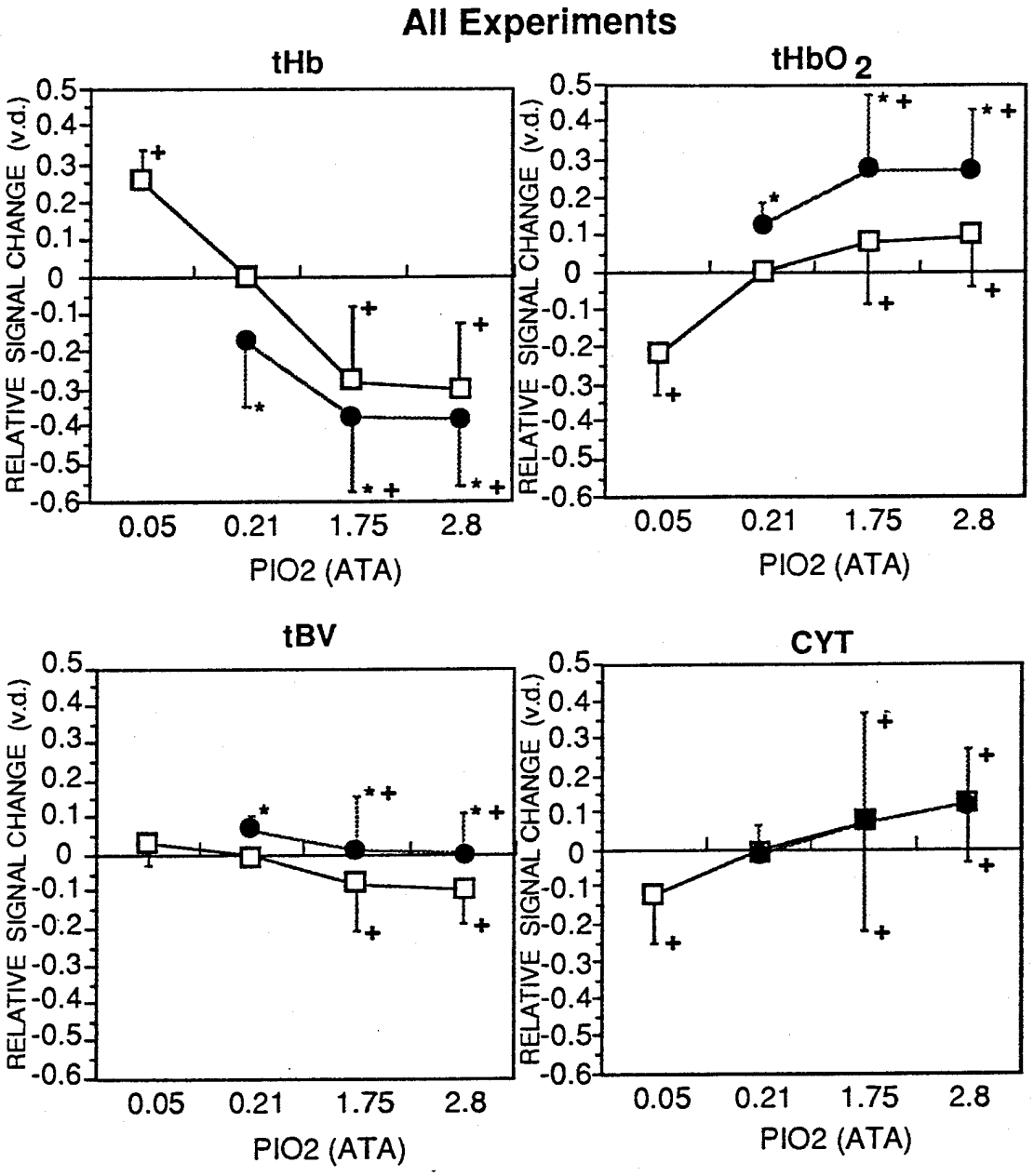

Figure 8: Mean cerebral oxygenation changes: All experiments. Changes in variations in density (v.d.) from normobaric, normoxic, normocapnic control in brain tHb, $tHbO_2$, tBV, and oxidized cytochrome a,a_3 in subjects breathing no CO_2 (□) and maximum CO_2, 60 torr PET_{CO_2}, (●) at different inspired oxygen pressures. Points at 0.05 ATA represent mean ± SD for 15 experiments. All other points represent mean ± SD for 30 experiments. * Significantly different from the no inspired CO_2 value, $P < 0.05$. + Significantly different from 0.21 ATA PI_{O_2} value, $P < 0.05$.

Rebreathing maneuvers resulting in progressive normocapnic hypoxia to 70% Sa_{O_2} (0.05 ATA PI_{O_2}), resulted in significant decreases in $tHbO_2$ ($p < .05$) and oxidized cytochrome a,a_3 ($p < .05$) and a significant increase in tHb ($p < .05$).

Rebreathing induced progressive hypercapnia resulted in significant decreases in tHb ($p < .05$) and significant increases in $tHbO_2$ ($p < .05$) and tBV ($p < .05$) at each PI_{O_2}, 0.21, 1.75, and 2.80 ATA O_2. The amount of oxidized cytochrome a,a_3 was statistically unchanged at all three PI_{O_2}'s with increased CO_2. tHb and tBV were significantly lower ($p < .05$) at PI_{O_2} = 1.75 and 2.80 ATA relative to the 0.21 ATA PI_{O_2} for the no CO_2 and maximum CO_2 conditions. There was significantly more $tHbO_2$ and oxidized cytochrome a,a_3 ($p <.05$) for the no CO_2 and high CO_2 conditons at increased PI_{O_2}'s relative to normoxia.

The possibility that the hypoxic stress had an effect on cerebral oxygenation measurements made in the later hypercapnic exposures was explored by removing the hypoxic exposure from the protocol for half of the 34 experiments. No statistically significant difference in cerebral oxygenation data was found between experiments with hypoxic exposures and those without.

Breakdown by Symptoms

Cerebral oxygenation data at maximum CO_2 were grouped according to symptoms. Data from the no inspired CO_2 conditions were not significantly altered by grouping and are not shown. Figures 9 to 11 compare the effects of increasing inspired oxygen pressure on the cerebral responses to maximum CO_2. Mean values for the same conditions in different groups are not statistically different using unpaired t-tests. Information comes from comparing trends as inspired oxygen increases for the responses at maximum CO_2 since all symptoms occurred near maximum CO_2 at 2.80 ATA PI_{O_2}. An ANOVA repeated measures test, Fisher PLSD, was used to make statistical determinations.

The group of experiments resulting in definite symptoms, n=6, showed no significant increase in $tHbO_2$ with increasing PI_{O_2} at maximum CO_2 relative to normoxia (Figure 9 - $tHbO_2$). Experiments with minor symptoms, n=13, showed significantly increased $tHbO_2$ (Figure 10 - $tHbO_2$) as did experiments with no reported symptoms, n=11, (Figure 11 - $tHbO_2$). tHb responses were the same for each group, a significant decrease with increasing PI_{O_2} at maximum CO_2 relative to normoxia (Figures 9-11 - tHb). The combination of these trends is indicated by the response of the brain tissue blood volume. A significant decrease in blood volume with increasing PI_{O_2} was observed for experiments with definite symptoms (Figure 9 - tBV), while the minor and no symptoms

groups showed no significant changes in blood volume responses at maximum CO_2 with hyperoxia (Figures 10 and 11 - tBV).

The minor symptom group showed significant increases in the amount of oxidized cytochrome a,a_3 at 1.75 and 2.80 ATA PI_{O_2} relative to 0.21 ATA PI_{O_2} (Figure 10 - CYT). The definite symptom group showed a significant increase at 1.75 ATA (Figure 9 - CYT). The asymptomatic group showed no significant change in the amount of oxidized cytochrome a,a_3 with increasing PI_{O_2} (Figure 11 - CYT).

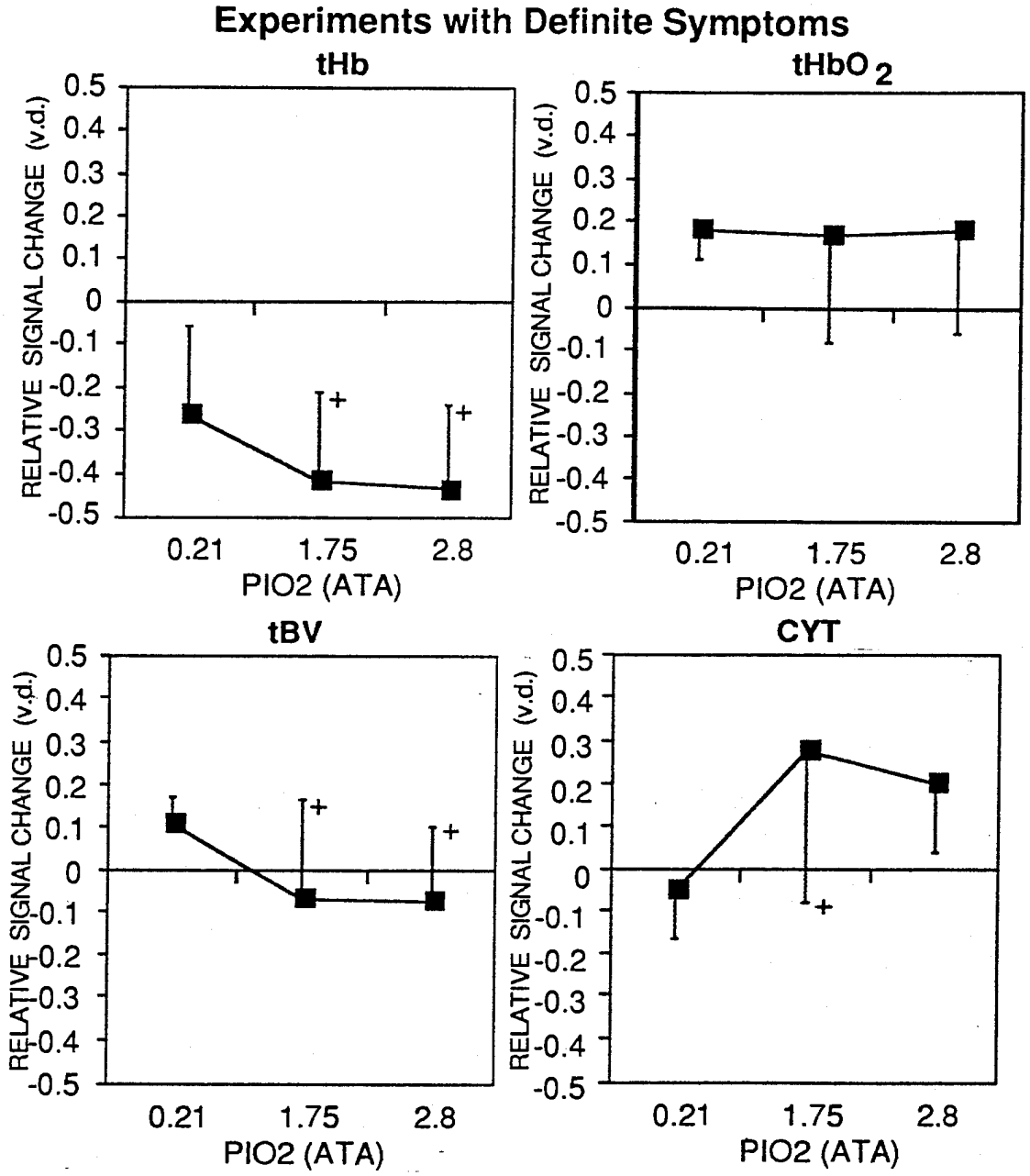

Figure 9: Mean cerebral oxygenation changes: Experiments with definite symptoms. Changes in variations in density (v.d.) from normobaric, normoxic, normocapnic control in brain tHb, $tHbO_2$, tBV, and oxidized cytochrome a,a_3 in subjects breathing maximum CO_2, 60 torr PET_{CO_2} (■) at 0.21, 1.75, and 2.80 PI_{O_2}. Points represent mean ± SD for 6 experiments resulting in reports of definite symptoms. + Significantly different from 0.21 ATA PI_{O_2} value, $P < 0.05$.

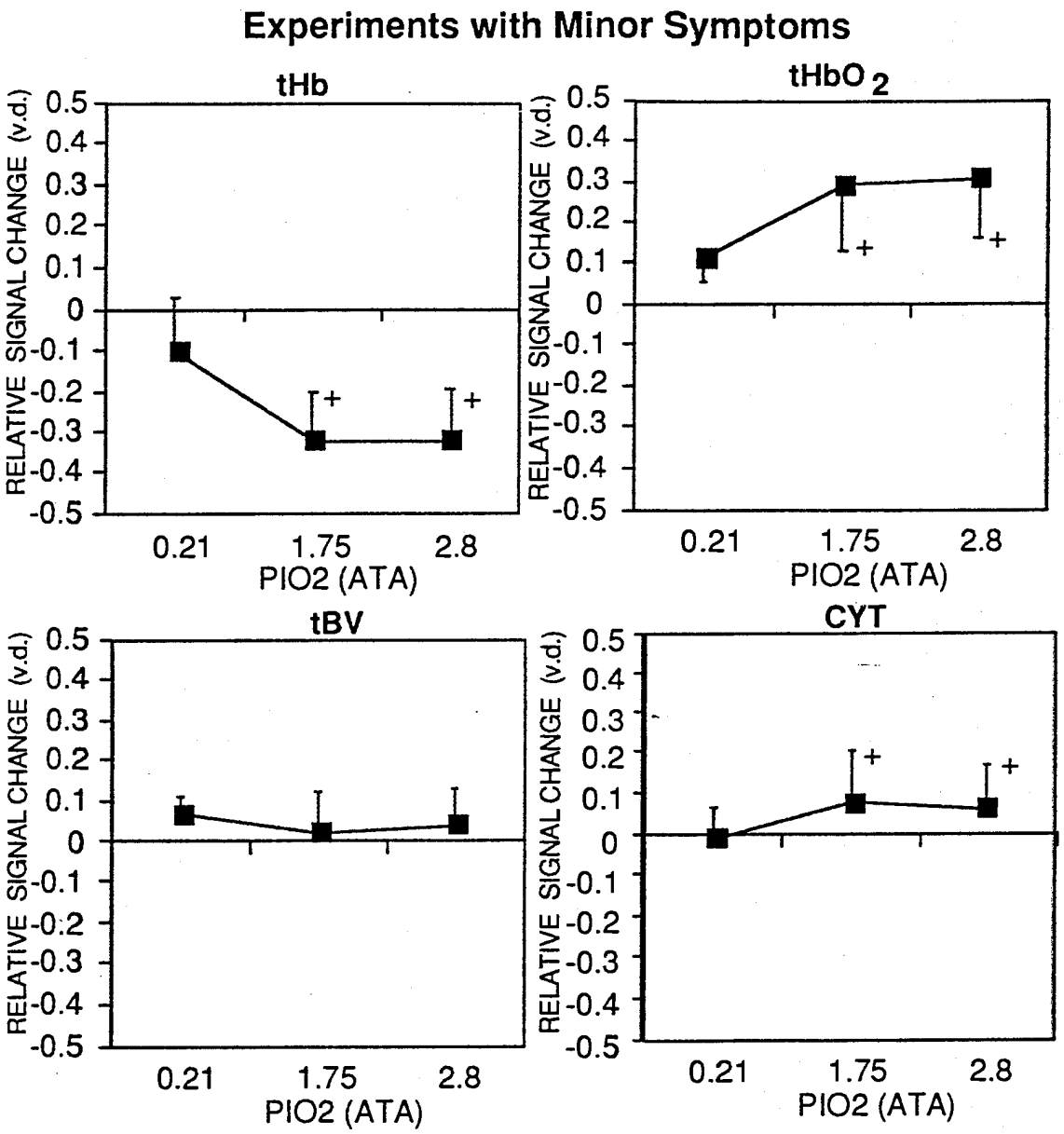

Figure 10: Mean cerebral oxygenation changes: Experiments with minor symptoms. Changes in variations in density (v.d.) from normobaric, normoxic, normocapnic control in brain tHb, $tHbO_2$, tBV, and oxidized cytochrome a,a_3 in subjects breathing maximum CO_2, 60 torr PET_{CO_2} (■) at 0.21, 1.75, and 2.80 PI_{O_2}. Points represent mean ± SD for 13 experiments resulting in minor symptoms. + Significantly different from 0.21 ATA PI_{O_2} value, $P < 0.05$.

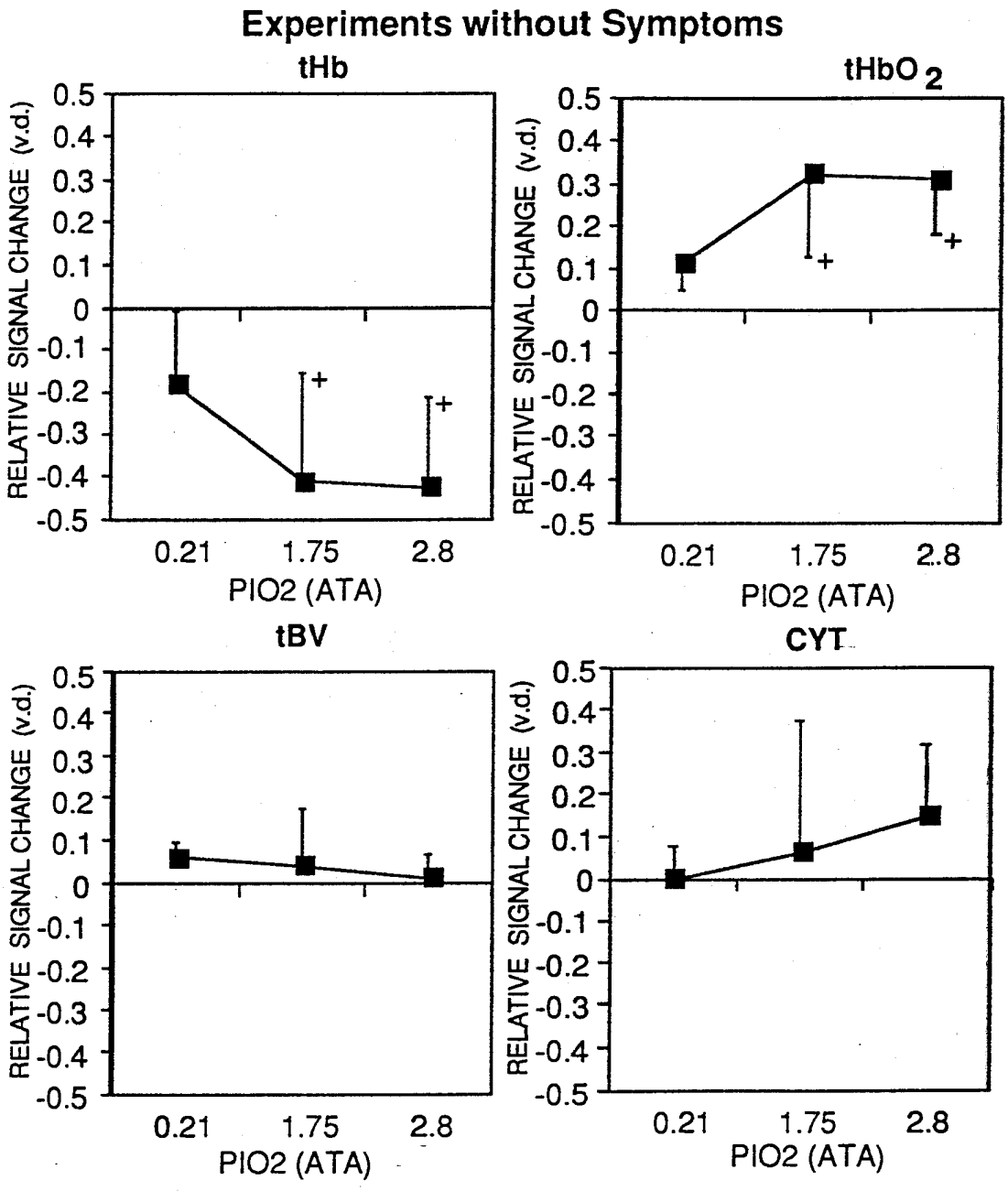

Figure 11: Mean cerebral oxygenation changes: Experiments without symptoms. Changes in variations in density (v.d.) from normobaric, normoxic, normocapnic control in brain tHb, $tHbO_2$, tBV, and oxidized cytochrome a,a_3 in subjects breathing maximum CO_2, 60 torr PET_{CO_2} (■) at 0.21, 1.75, and 2.80 PI_{O_2}. Points represent mean ± SD for the 11 experiments resulting in no reported symptoms. + Significantly different from 0.21 ATA PI_{O_2} value, $P < 0.05$.

Ventilatory Response

The ventilatory response data suggest that individuals with depressed CO2 response during hyperoxia may be more susceptible to CNS oxygen toxicity. All ventilatory response data are listed in tabular form in Appendix 2. No statistically significant difference in ventilatory response data was found between experiments with hypoxic exposures and those without. Therefore, mean ± standard deviation for ventilatory response slopes measured from experiments with and without hypoxic exposures are included in the results, n = 30 (Figure 12). Four experiments were not included due to data loss from computer failure or incomplete testing. Two of these experiments had reported minor symptoms, one had a report of a definite symptom, and one had no reported symptoms.

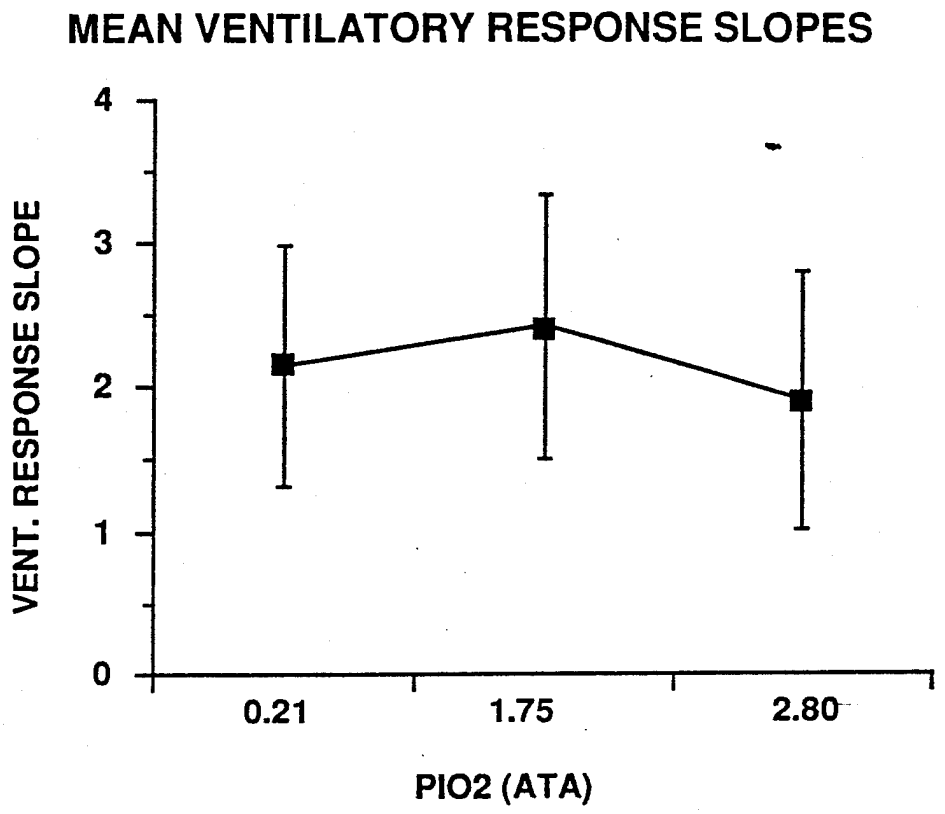

Figure 12: Mean ventilatory response slopes, ± SD, ($lpm \cdot torr^{-1}$) at 0.21, 1.75, and 2.80 ATA PI_{O_2} for 30 experiments.

Subject Variability

Variability of ventilatory response slopes measured for the same subject under identical conditions at different times is measured using the coefficient of variability. Table 5 shows within subject variability at each PI_{O_2} tested.

Table 5

Ventilatory Response Slope Coefficients of Variation for Repeated Experiments

Subject	N	0.21 ATA PI_{O_2} %	1.75 ATA PI_{O_2} %	2.80 ATA PI_{O_2} %
AE	4	24.9	17.8	36.2
CM	8	29.1	22.5	31.6
CP	2	7.1	1.8	13.8
DS	3	20.4	22.1	23.7
DV	3	8.2	22.4	27.2
OD	5	45.6	31.9	17.1
TA	3	18.1	36.1	21.1

The mean, ± SD, time required for PET_{CO_2} to increase from normocapnia to 60 torr at each PI_{O_2} were:

0.21 ATA PI_{O_2} 4.8 ± 1.1 min.
1.75 ATA PI_{O_2} 8.6 ± 2.3 min.
2.80 ATA PI_{O_2} 5.3 ± 1.2 min.

Effect of Hyperoxia

Some subjects exhibited depressed mean ventilatory response slopes with hyperoxia (ventilatory response normalized to normoxic response < 1) while others showed increased ventilatory responses with increased oxygen (Figure

13). All but one subject showed lower response to CO_2 at 2.80 ATA PI_{O_2} than at 1.75 ATA PI_{O_2}.

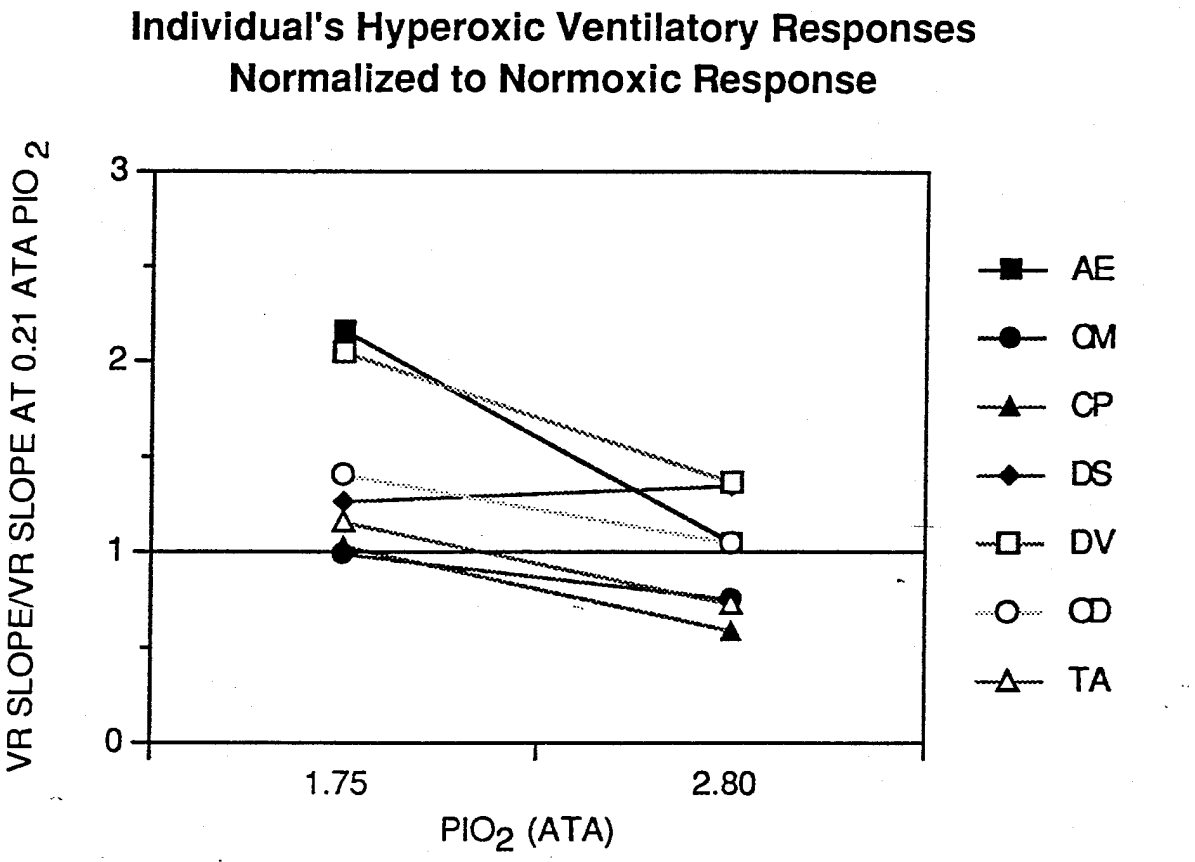

Figure 13: Individual's hyperoxic ventilatory response slopes normalized to normoxic responses at 1.75, and 2.80 ATA PI_{O_2} for subjects that participated in more than one experiment. AE - n=4, CM - n=8, DS - n=3, RV - n=3, TA - n=4, CP - n=2, OD - n=5.

Breakdown by Symptoms

When experiments with definite symptoms are separated out, ventilatory response slopes at 2.80 ATA PI_{O_2} were lower than those measured at 0.21 ATA PI_{O_2} (Figure 14). The line drawn is a line of identity indicating no difference in

ventilatory response. If the minor symptoms are considered, 9 out of 12 fall below the line of identity. Only 3 of 12 in the asymptomatic group fall below this line.

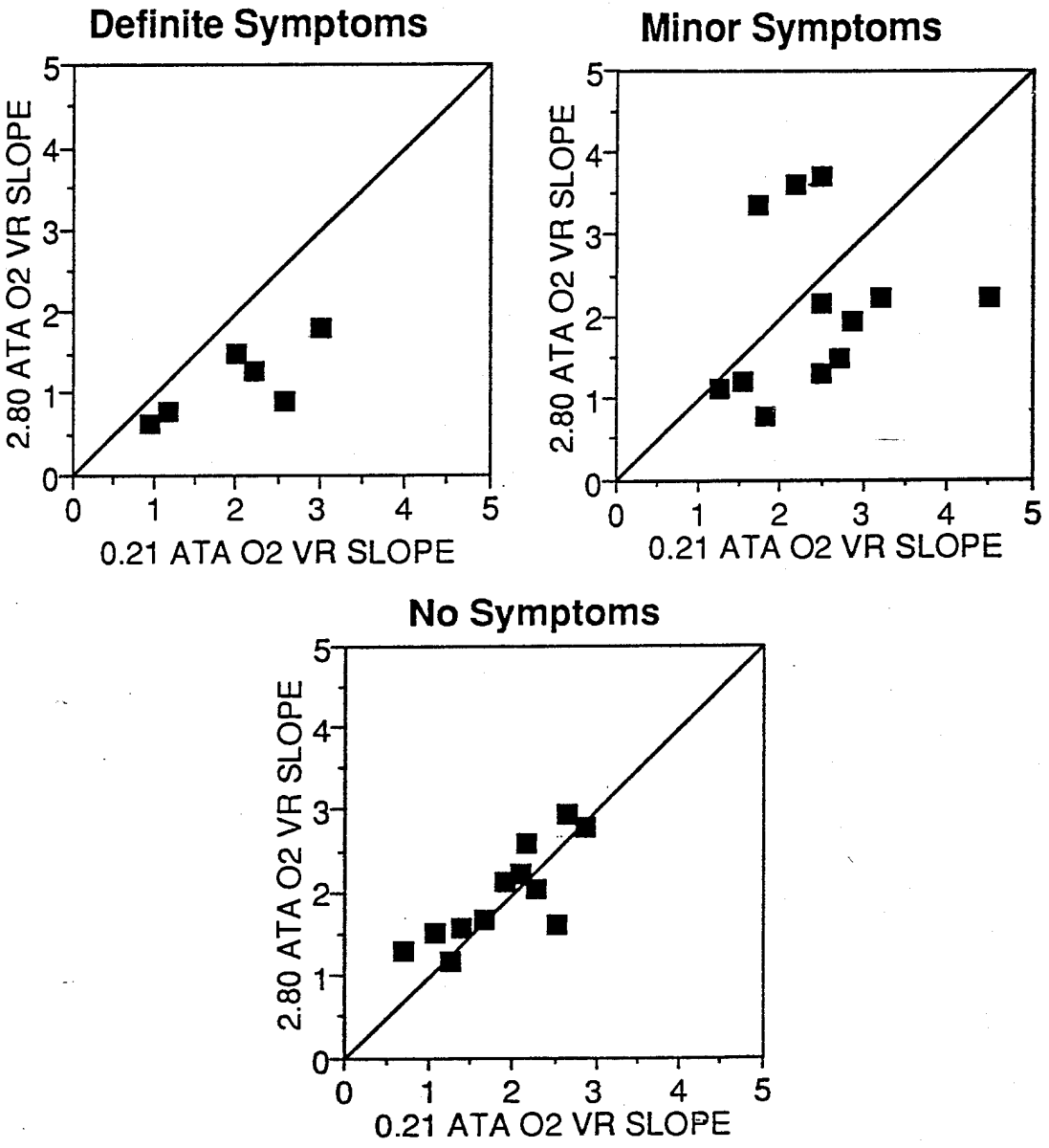

Figure 14: Ventilatory response slopes: Effect of PI_{O_2} in relation to symptoms. Ventilatory response slopes ($lpm \cdot torr^{-1}$) were grouped as experiments with reports of definite symptoms, any symptoms (definite and minor), and no symptoms.

When the hyperoxic ventilatory responses are normalized to normoxic responses, the mean responses from experiments resulting in reported symptoms, definite or minor, are significantly lower at 2.80 ATA PI_{O_2} than at 1.75 ATA PI_{O_2} while the asymptomatic group's responses are not (Figure 15). At 2.80 ATA PI_{O_2}, the responses from experiments with reported symptoms were significantly lower than those without reported symptoms. Subjects with mean decreases in ventilatory response at 2.80 PI_{O_2} ATA relative to normoxia for repeated experiments did not always exhibit symptoms.

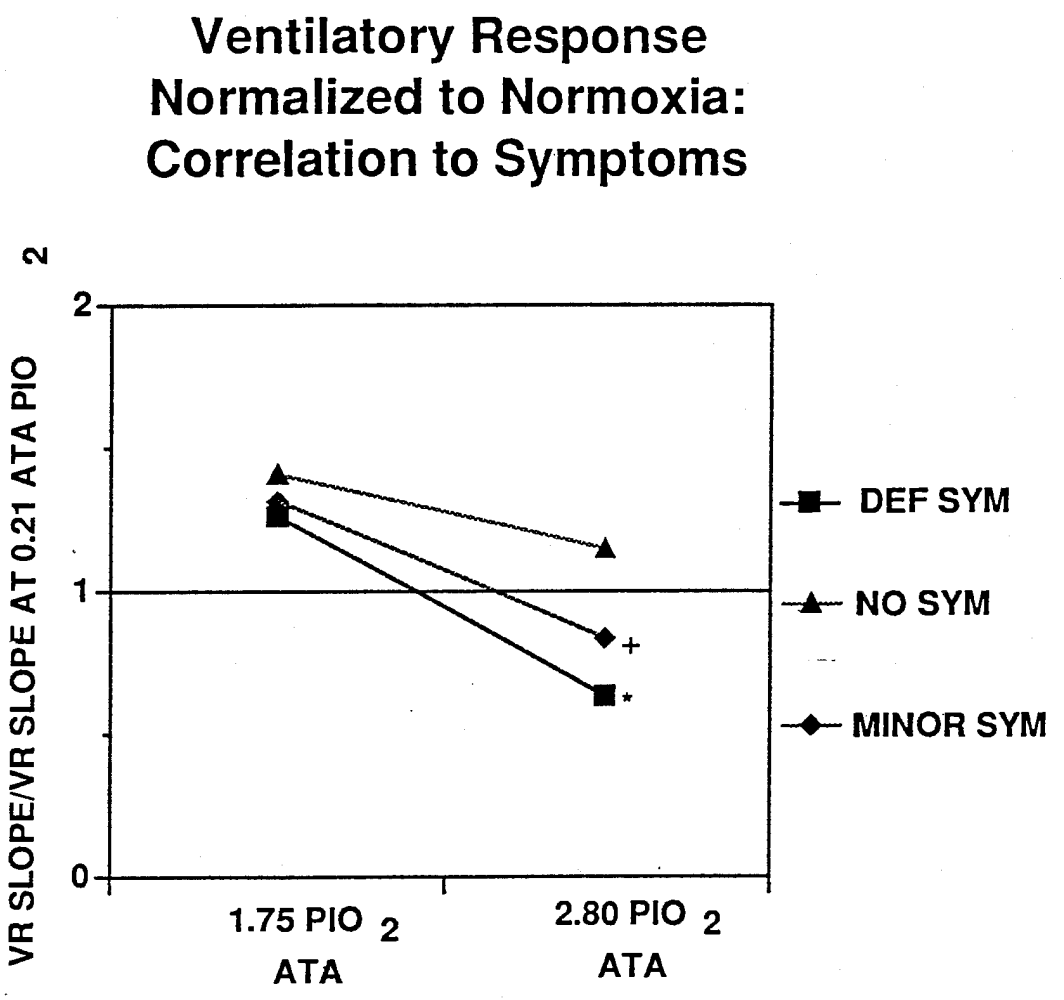

Figure 15: Ventilatory response slopes normalized to 0.21 ATA PI_{O_2} : Correlation to symptoms. Normalized ventilatory response slopes were grouped as experiments with reports of definite symptoms, any symptoms (definite and minor), and no symptoms. DEF SYM - n=6, ALL SYM - n=12, and NO SYM - n=12 + Significantly different from values at 1.75 ATA PI_{O_2}, $P < 0.05$ * Significantly different from corresponding no symptoms value, $P < 0.05$

5. DISCUSSION

Symptoms

All symptoms occurred during the conditions of the experimental protocol that maximized oxygen delivery to the brain, i.e., 2.80 ATA PI_{O_2} and 60 Torr PET_{CO_2}. Previous hypoxic exposure did not have a significant effect on symptom incidence. Symptoms were divided into two groups, based on classifications proposed by Butler and Thalmann (1984), definite (tinnitus, tunnel vision, disorientation, muscle twitching, and incoordination) and minor or probable (light-headedness, apprehension, dysphoria, and lethargy) . Butler and Thalmann (1984) also classified probable symptoms as definite if they were severe. In our experiments, this applied to extreme anxiety, which was accompanied by a feeling of impending unconsciousness. Our other definite symptoms, tinnitus and tunnel vision, agreed with Butler and Thalmann's classification. Our minor symptoms were similar to Butler and Thalmann's probable symptoms with the exception of sweats, numbness, tingling, and narcosis. These are not typical symptoms of CNS oxygen toxicity and may have arisen from the combination of oxygen and carbon dioxide stressors. The ambiguity of these symptoms caused us to use the term minor instead of probable.

Cerebral Oxygenation

Hypoxia to 70% Sa_{O_2} produced similar NIR signal changes to those measured in an earlier study with the same NIRS instrument (Hampson et al. 1990). A previous hypoxic exposure did not have a significant effect on later hypercapnic exposures.

Cerebral vasoconstriction in response to elevated PI_{O_2} and vasodilation in response to hypoxia are evident from measurements taken during normocapnic breathing (Figure 8 - tBV - □). Vasoregulation is affected by O_2 and CO_2 tension. The mechanism by which oxygen exerts its regulatory effect is unknown (Jackson 1987). Cerebral vasodilation in response to hypoxia is generally accepted to be mediated locally (Traystman and Fitzgerald 1981) as is vasoconstriction in response to hyperoxia since oxygen decreases vessel lumen size *in vitro* (Plewes and Farhi 1983).

Arterial hypocapnia causes vasoconstriction and hyperbaric oxygen decreases Pa_{CO_2} and arterial $[H^+]$. Hyperoxia increases the amount of oxygen dissolved in plasma. More hemoglobin remains oxygenated than during normoxia because dissolved oxygen is preferentially utilized by the tissues. During air breathing, O_2 is released from hemoglobin and deoxygenated hemoglobin (Hb) is reduced during the dissociation of carbonic acid into hydrogen ions and bicarbonate, the form in which most CO_2 is transported. CO_2

is also transported via carbamino compounds, which are more readily formed by Hb than HbO_2. During hyperoxia, hemoglobin reduction is decreased and the amount of HbO_2 increased (Salzano 1980). Therefore, hemoglobin does not fulfill its normal role in CO_2 transport, increasing Pt_{CO_2} up to 5 torr above normal and decreasing Pa_{CO_2} which causes vasoconstriction (Lambertsen 1978). In our study, the increase in HbO_2 and decrease in Hb and blood volume with hyperoxia are evident (Figure 8 - tHb, $tHbO_2$, and tBV - □).

CO_2 is a powerful cerebral vasodilator. CO_2 produces vascular changes by altering perivascular and intracellular smooth muscle pH, although the mechanism of vasoregulation by pH and CO_2 is unclear (Berne 1986). Lambertsen (1955) showed that cerebral venous oxygen content remained constant for PI_{O_2}'s of 0.21 or 3.5 ATA, but when PI_{CO_2} was raised to 53 torr, the venous oxygen content increased by 1000% drastically elevating cerebral oxygenation. Cerebral oxygen metabolism is maintained at a constant level for Pt_{O_2}'s from 50 torr to those which precipitate oxygen toxicity (Lambertsen 1978). Cerebral metabolic oxygen rate is maintained via balancing of cerebral blood flow, which is directly related to blood volume, and cerebral arteriovenous (AV) oxygen difference (Piantadosi 1989). Addition of CO_2 decreases AV O_2 difference and increases cerebral blood flow via vasodilation. These effects are seen in Figure 8 (tBV -

●). At maximum PET_{CO_2}, 60 torr, blood volume was significantly higher than during normocapnia for each PI_{O_2}.

Hypercapnic induced increases in Pa_{CO_2} and $[H^+]$ reverse oxygen related vasoconstriction . Torbati (1987) proposed that the CNS limits for oxygen tolerance may be determined by the ability of cerebral vessels to maintain vasoconstriction during hyperoxia. In animals, the vasoconstrictive effect of oxygen fails before onset of seizures (Bean, Lingnell, and Burgess 1972). Release of vasoconstriction increases blood flow and Pt_{O_2} and may precipitate CNS oxygen toxicity due to increased generation of reactive oxygen species (McCord and Fridovich 1978).

In our study, all experiments showed vasoconstriction without CO_2 and vasodilation at maximum PET_{CO_2}, 60 torr, during hyperoxia, but the degree of the vascular changes with increasing PI_{O_2} differed between experiments with definite symptoms and and those without symptoms. Experiments with minor symptoms appeared to exhibit significant trends common to both other groups. The ambiguity of the symptoms prevent their inclusion with the other groups. Experiments with definite symptoms had significantly reduced blood volume at maximum CO_2 at 1.75 and 2.80 ATA PI_{O_2} relative to normoxia.

Reduced vasodilation or maintenance of oxygen induced vasoconstriction would tend to limit oxygen delivery. Definite symptoms would be expected to occur during increased oxygen delivery according to the working hypothesis. The

apparent discrepency in our results may be due to the fact that we are monitoring a small sample of a heterogeneous organ (Bean 1961). A range of P_{O_2}'s, 1-90 torr, have been found simultaneously in different areas of the brain (Lubbers and Starlinger 1975). Increases in blood volume may have occurred in other areas of the brain, where conditions of increased oxygenation may have precipitated toxicity and thus the appearance of symptoms. Alternatively, the reduced vasodilatory response may have been mediated by the development of oxygen toxicity. Increased formation of oxygen radicals attributed to causing oxygen toxicity may cause a vasoconstriction which overrides the effect of increased CO_2.

In our study, the amount of oxidized cytochrome a,a_3 increased during normocapnic hyperoxia at 1.75 and 2.80 ATA PI_{O_2}, while normocapnic hypoxia to 70% Sa_{O_2} had the opposite effect (Figure 8 - CYT - □). The redox state of cytochrome a,a_3 is an indicator of cerebral oxidative metabolism and is a nearly continuous function of oxygen concentration from hypoxia to normoxia (Hampson et al. 1990). The amount of cytochrome a,a_3 that is normally oxidized is not known although studies in anesthetized animals suggest cytochrome a,a_3 is 54% oxidized in normoxia (Sylvia et al. 1984) while *in vitro* studies have shown nearly complete oxidation of cytochrome a,a_3 (Chance and Williams 1956). Cytochrome a,a_3 oxidation has been shown to increase slightly with hyperoxia

in anesthetized animals (Hempel et al. 1977). Increases in cytochrome oxidation with hyperoxia may indicate increased cerebral metabolism although studies have shown cerebral ATP levels remain constant during hyperbaric oxygenation (Nolan and Faiman 1974). Oxidation of NADH and increased electron flow rate through the respiratory chain has been found during hyperbaric oxygen exposure (Chance et al. 1965).

In our study, cytochrome a,a3 oxidation did not change significantly at maximum CO_2, 60 torr PET_{CO_2}, despite increased oxygen delivery to the brain as a result of CO_2 induced vasodilation (Figure 8 - CYT - ●). This suggests metabolic regulation of the mitochondrial redox state at the site monitored in awake humans. Vasoregulation appears to be sufficient to maintain normal cerebral oxidative metabolism to 2.80 ATA PI_{O_2} and 60 torr PET_{CO_2} when symptoms of oxygen toxicity are not present. Increased oxidation of cytochrome a,a3 due to hyperoxic hypercapnia has been found in anesthetized animals at 55 torr PI_{CO_2} (Hempel et al. 1977). The disparity between this study and ours may be due to differences between anesthetized animals and awake humans or differences in spectrophotometric methods.

Although cytochrome a,a3 oxidation was not increased by addition of CO_2, the degree of oxidation of cytochrome a,a3 at maximum CO_2, 60 torr PET_{CO_2}, increased at 1.75 but not at 2.80 ATA PI_{O_2} compared to 0.21 ATA PI_{O_2} for experiments with definite symptoms. Experiments with no symptoms resulted in

no significant increases in oxidized cytochrome with hyperoxia (Figure 11 - CYT). Increased cytochrome oxidation in the symptomatic group at 2.80 ATA O_2 and maximum CO_2 would be consistant with the hypothesis that increased oxygen delivery increases the likelihood of oxygen toxicity formation. This was not the case in these experiments and could be attributed to the monitoring of an unrepresentative area of the brain, while increased oxygen delivery occurred elsewhere. The increased cytochrome a,a_3 oxidation at 1.75 ATA PI_{O_2} in subjects with definite symptoms is curious and may have been due to the increased cerebral metabolism after symptom occurrence.

NIRS is a capable method for monitoring cerebral oxygenation parameters but has not shown direct correlations between the development of CNS oxygen toxicity symptoms or the susceptibility of an individual to CNS oxygen toxicity and cerebral oxygenation changes at the site monitored.

Ventilatory Response

Our rebreathing method differed from clinical methods, in which rebreathing begins with 7% CO_2, nearly matching normal Pv_{CO_2} at 50 torr (Read 1967). In clinical studies, ventilatory response to CO_2 ranged from 0.56 to 8.16 lpm $Torr^{-1}$, in healthy subjects, although 80% had responses between 1.00 and 4.00 $lpm \cdot torr^{-1}$ (Rebuck and Read 1971). Sex, genetic factors, personality, lung size, and mental

activity affect ventilatory response. (Rebuck and Slusky 1981). The coefficient of variation of ventilatory response for a subject tested repeatedly, 2 - 6 times, on the same day ranged from 2 to 50%.

We obtained similar results in our study, despite methodological differences. In our study, ventilatory responses ranged from 0.66 to 6.07 $lpm \cdot torr^{-1}$ with 88% of responses between 1.00 and 4.00 $lpm \cdot torr^{-1}$. In repeat experiments, 2 to 8 times, weeks or months apart, we noted coefficients of variation ranging from 2 to 46%.

Hyperoxia increases dissolved oxygen content while hemoglobin remains saturated, reducing its effectiveness as an aid in the transport of CO_2 to the lungs. This raises P_{tCO_2} up to 5 torr over normoxia, increasing ventilation slightly. Hyperoxia also depresses central respiratory control leading to reduced response to CO_2 (Lambertsen 1978). For the overall group of experiments, we noted reduced ventilatory responses at 2.80 ATA PI_{O_2} but increased responses at 1.75 ATA PI_{O_2} compared to normoxia (Figure 12). The cause of the larger ventilatory response slopes at 1.75 ATA PI_{O_2} is unknown but may have been due to increased time needed to achieve 60 Torr PET_{CO_2} as a result of nitrogen elimination associated with decompression from 77 to 35 fsw. Increased duration could cause apprehension thus increasing breathing frequency. Also, the 1.75 ATA PI_{O_2} exposure was the last of the experiment and discomfort caused by the optrodes was at

its maximum, potentially causing increased breathing frequency. New studies are underway to better assess the cause of the increased ventilatory responses in hyperoxia.

In experiments with symptoms, ventilatory response was decreased at 2.80 ATA PI_{O_2} (Figure 15). Depressed ventilatory response increases cerebral Pt_{CO_2}, cerebral vasodilation, cerebral oxygenation and risk of CNS oxygen toxicity (Lanphier 1975). These data suggest that susceptibility to CNS oxygen toxicity symptoms may be related to CO_2 retention and responses by an individual are dynamic.

6. CONCLUSIONS

NIR spectroscopy is suitable for noninvasively monitoring cerebral oxygenation in awake humans. The vasoconstrictive effect of increased PI_{O_2} was demonstrated in exposures to 1.75 and 2.80 ATA of oxygen. CO_2 rebreathing leading to hypercapnia reversed this effect. The use of NIRS during hyperoxic hypercapnia indicates normal regulation of cytochrome a,a_3 oxidation at 2.80 ATA PI_{O_2} and 60 Torr PET_{CO_2}. Symptoms of CNS oxygen toxicity may occur without reversal of hyperoxic cerebral vasoconstriction during CO_2 rebreathing. Ventilatory response measurements suggest that individuals with depressed CO_2 response during hyperoxia may be more susceptible to CNS oxygen toxicity.

The results of this study support the hypothesis that inspired CO_2 increases the likelihood that symptoms of CNS oxygen toxicity will develop by increasing cerebral oxygenation, but do not provide definitive proof. The study was not able provide a means of predicting the onset of CNS oxygen toxicity via monitoring of cerebral oxygenation and ventilation. The results of this study support the hypothesis that monitoring of changes in cerebral oxygenation and ventilation may provide a means of determining an individual's susceptibility to CNS oxygen toxicity. However, further testing of individuals with a known susceptibility to

CNS oxygen toxicity is required to provide definitive support for the hypothesis.

Further studies will investigate the hyperoxic-hypercapnic stresses of immersion, extended duration, and exercise in an effort to shed more light upon the development of CNS oxygen toxicity symptoms.

APPENDIX 1: CEREBRAL OXYGENATION DATA

This appendix contains cerebral oxygenation data obtained via NIR Spectroscopy. The data is presented as differences from signals obtained during the control period of the experiment, normobaric, normoxic, normocapnic gas breathing. N/A appears under 0.05 ATA PIO_2 for those experiments without a hypoxic exposure. The results from 4 incomplete experiments are not included.

DEOXYGENATED HEMOGLOBIN (tHb)

P_{IO_2}	0.05 ATA	0.21 ATA	1.75 ATA	1.75 ATA	2.80 ATA	2.80 ATA
P_{ETCO_2}		60 torr		60 torr		60 torr
SUBJECT						
DV	0.2759	-0.0702	-0.2225	-0.2830	-0.2116	-0.3086
TA	0.1748	-0.0617	-0.1893	-0.2547	-0.1601	-0.2688
DV	0.2376	-0.0377	-0.1188	-0.2626	-0.1731	-0.3076
MN	0.4198	-0.0578	-0.1708	-0.3070	-0.2418	-0.3364
TA	0.2698	-0.0522	-0.3367	-0.4976	-0.3611	-0.4679
CM	0.2716	-0.0767	-0.3141	-0.5042	-0.4808	-0.5062
DV	0.3774	-0.0287	-0.2965	-0.3509	-0.2757	-0.3186
CM	0.2333	-0.0973	-0.1148	-0.2233	-0.1347	-0.1858
OD	0.1992	-0.0092	-0.1232	-0.1852	-0.1123	-0.1828
MN	0.1507	-0.0209	-0.0932	-0.1840	-0.0507	-0.1283
CM	0.1786	-0.0732	-0.2567	-0.3048	-0.2335	-0.2560
OD	0.2959	-0.0505	-0.0648	-0.1419	-0.1439	-0.1894
CP	0.3465	-0.1051	-0.1694	-0.2508	-0.1483	-0.2300
DS	N/A	-0.0003	-0.1224	-0.2251	-0.1977	-0.2452
DS	N/A	-0.0202	-0.1683	-0.3107	-0.2007	-0.3130
OD	N/A	-0.0487	-0.0155	-0.0597	-0.2479	-0.1363
CM	N/A	-0.0809	-0.2043	-0.3153	-0.2282	-0.2656
OD	N/A	-0.0084	-0.5554	-0.497	-0.3237	-0.4787
CM	N/A	-0.1159	-0.0738	-0.3316	-0.1122	-0.2206
AE	N/A	-0.0706	-0.2205	-0.3020	-0.2026	-0.3010
CM	N/A	-0.0433	-0.1174	-0.2420	-0.1824	-0.2603
AE	N/A	-0.0600	-0.2147	-0.3479	-0.3076	-0.3905
SH	N/A	-0.1088	-0.2616	-0.1629	-0.1239	-0.1524
AE	N/A	-0.0705	-0.1603	-0.3614	-0.1993	-0.3928
CM	N/A	-0.0302	-0.1307	-0.3508	-0.1415	-0.2148
CM	N/A	-0.0436	-0.1481	-0.2574	-0.2226	-0.2476
OD	0.1579	-0.0503	-0.1045	-0.1173	-0.2003	-0.3114
DS	0.288	-0.0265	-0.0126	-0.1777	-0.0664	-0.1839
TS	0.193	-0.0477	0.0092	-0.071	-0.0958	-0.1946
CP	N/A	-0.0106	0.07410	-0.0021	-0.0487	-0.1027

OXYGENATED HEMOGLOBIN ($tHbO_2$)

PIO_2	0.05 ATA	0.21 ATA	1.75 ATA	1.75 ATA	2.80 ATA	2.80 ATA
$PETCO_2$		60 torr		60 torr		60 torr
SUBJECT						
DV	-0.2261	0.1184	0.1807	0.2918	0.1997	0.3473
TA	-0.1516	0.1090	0.0650	0.1681	0.0434	0.2534
DV	-0.1560	0.0596	-0.0573	0.1221	0.1133	0.2765
MN	-0.4437	0.1494	0.0232	0.2961	0.1427	0.3345
TA	-0.3211	0.1418	0.2319	0.5591	0.2642	0.6915
CM	-0.1971	0.2915	0.2252	0.5009	0.4273	0.5651
DV	-0.2758	0.0647	0.1254	0.2569	0.1864	0.4022
CM	-0.1866	0.1788	0.0494	0.2865	0.0627	0.1958
OD	-0.2034	0.0215	0.0934	0.1925	0.0897	0.1763
MN	-0.0777	0.1343	0.0697	0.221	-0.0203	0.0992
CM	-0.1725	0.2266	0.1643	0.5315	0.1646	0.4557
OD	-0.2684	0.0972	-0.1493	0.0097	-0.0886	0.0504
CP	-0.4269	0.2060	-0.3317	-0.2402	-0.3190	-0.1551
DS	N/A	0.0450	-0.0200	0.1294	0.1685	0.2849
DS	N/A	0.0400	0.1377	0.306	0.1693	0.2756
OD	N/A	0.1115	0.1150	0.2366	0.1915	0.2182
CM	N/A	0.1658	0.0707	0.5019	0.1071	0.2585
OD	N/A	0.1111	0.4506	0.4659	0.2787	0.4373
CM	N/A	0.2433	-0.0668	0.5108	-0.1434	0.1569
AE	N/A	0.1558	0.1329	0.3012	0.0611	0.2400
CM	N/A	0.0798	0.0396	0.3554	0.0081	0.2683
AE	N/A	0.1165	0.1255	0.3264	0.2646	0.4355
SH	N/A	0.2470	0.4823	0.2128	0.0492	0.1693
AE	N/A	0.1507	0.0577	0.4603	0.1298	0.5016
CM	N/A	0.0304	0.2800	0.6526	0.0866	0.2463
CM	N/A	0.1318	0.0505	0.3531	0.1108	0.2919
OD	-0.0984	0.2223	0.1448	0.211	0.3491	0.4263
DS	-0.1441	0.0393	-0.1503	0.0293	-0.0818	0.0876
TS	-0.1359	0.1036	-0.0641	0.0654	0.0633	0.2620
CP	N/A	0.0819	-0.0733	0.019	0.0902	0.2002

BLOOD VOLUME (tBV)

P_{IO_2}	0.05 ATA	0.21 ATA	1.75 ATA	1.75 ATA	2.80 ATA	2.80 ATA
P_{ETCO_2}		60 torr		60 torr		60 torr
SUBJECT						
DV	0.0496	0.0480	-0.0419	0.0087	-0.0120	0.0385
TA	0.0232	0.0471	-0.1243	-0.0866	-0.1167	-0.0194
DV	0.0815	0.0219	-0.176	-0.1404	-0.0597	-0.031
MN	-0.0240	0.0917	-0.1477	-0.011	-0.0991	-0.0019
TA	-0.0148	0.0896	-0.1047	0.0615	-0.0969	0.2235
CM	0.0745	0.2148	-0.0889	-0.0033	-0.0527	0.0589
DV	0.1016	0.0361	-0.1710	-0.0938	-0.0892	0.0837
CM	0.0467	0.0816	-0.0654	0.0632	-0.0720	0.0099
OD	-0.0042	0.0122	-0.0297	0.0073	-0.0226	-0.0066
MN	0.0730	0.1134	-0.0235	0.0362	-0.0710	-0.0291
CM	0.0069	0.1542	-0.0915	0.2275	-0.0680	0.2005
OD	0.0275	0.0467	-0.2140	-0.1322	-0.2325	-0.1389
CP	-0.0805	0.1009	-0.5010	-0.4909	-0.4672	-0.3850
DS	N/A	0.0446	-0.1423	-0.0957	-0.0292	0.0397
DS	N/A	0.0199	-0.0305	-0.0047	-0.0314	-0.0374
OD	N/A	0.0627	0.0994	0.1767	-0.0564	0.0819
CM	N/A	0.0848	-0.1336	0.1865	-0.1211	-0.0070
OD	N/A	0.1026	-0.1049	-0.0311	-0.0451	-0.0415
CM	N/A	0.1273	-0.1406	0.1790	-0.2556	-0.0637
AE	N/A	0.0851	-0.0876	-0.0009	-0.1415	-0.0610
CM	N/A	0.0365	-0.0778	0.1135	-0.1743	0.0080
AE	N/A	0.0566	-0.0892	-0.0214	-0.0429	0.0450
SH	N/A	0.1382	0.2207	0.0498	-0.0746	0.0168
AE	N/A	0.0803	-0.1027	0.0989	-0.0695	0.1087
CM	N/A	0.0003	0.1493	0.3018	-0.0549	0.0314
CM	N/A	0.0883	-0.0975	0.0957	-0.1117	0.0449
OD	-0.0667	0.0456	-0.0859	-0.0327	0.0224	-0.0115
DS	0.1438	0.0129	-0.1630	-0.1484	-0.1481	-0.0954
TS	0.0570	0.0559	0.0352	-0.0055	-0.0324	0.0674
CP	N/A	0.0713	0.0007	0.0168	0.0415	0.0975

CYTOCHROME a, a_3 (CYT)

P_{IO_2}	0.05 ATA	0.21 ATA	1.75 ATA	1.75 ATA	2.80 ATA	2.80 ATA
P_{ETCO_2}		60 torr		60 torr		60 torr
SUBJECT						
DV	-0.0909	-0.0047	0.0331	0.0197	0.0745	0.1029
TA	-0.0762	-0.0284	0.1403	0.1365	0.1358	0.0878
DV	-0.0954	0.0308	0.1426	0.2016	0.0900	0.1685
MN	0.0979	-0.1141	0.1064	0.1231	0.0533	0.0478
TA	-0.1985	-0.0162	0.3781	0.1253	0.4675	-0.1393
CM	-0.5313	0.1243	0.3483	0.4622	0.2534	0.1153
DV	-0.2863	0.0270	-0.1207	0.1412	-0.0788	-0.0495
CM	-0.145	-0.0306	0.0654	0.0316	0.0887	0.0721
OD	-0.0034	0.1334	0.0730	0.0834	0.0668	0.0730
MN	-0.2120	-0.1407	-0.0147	0.0340	-0.0289	0.0340
CM	-0.3765	-0.0614	0.0090	-0.1952	0.0902	-0.0764
OD	-0.0577	-0.1025	0.2429	0.1775	0.3281	0.2439
CP	0.1097	-0.1503	0.7504	0.9140	0.4001	0.4665
DS	N/A	0.0231	0.0787	0.0975	0.2495	0.1575
DS	N/A	0.0559	0.0591	0.1614	0.1019	0.1905
OD	N/A	0.0108	0.2159	0.1538	0.3071	0.2196
CM	N/A	-0.0526	-0.0274	-0.1221	0.1299	0.1811
OD	N/A	-0.0498	0.4166	0.4237	0.2087	0.3490
CM	N/A	-0.1719	0.0868	-0.2841	0.1896	0.1251
AE	N/A	-0.0004	-0.0878	-0.0207	0.1072	0.1819
CM	N/A	0.1168	0.1115	0.1491	0.0677	0.1683
AE	N/A	-0.0006	-0.3559	-0.0836	-0.0448	0.0339
SH	N/A	-0.1790	-0.4503	-0.0192	0.0807	-0.0181
AE	N/A	-0.0715	0.1772	-0.0808	0.2263	-0.0986
CM	N/A	0.0750	-0.3330	-0.5157	-0.0051	-0.0198
CM	N/A	0.0153	0.0742	-0.0225	0.1615	0.1806
OD	-0.0898	0.0360	0.5982	0.5388	0.4461	0.4968
DS	-0.2227	0.0201	0.2350	0.4346	0.1438	0.2298
TS	-0.1600	0.0047	-0.0494	-0.0363	-0.0610	-0.0167
AE	-0.1530	0.1404	-0.7265	-0.7265	-0.3149	-0.3843
CP	N/A	0.0804	0.2160	0.2633	0.0403	0.1576

APPENDIX 2: VENTILATORY RESPONSE DATA

This appendix contains ventilatory response data obtained during the progressive hypercapnia exposures. Data are presented as slopes ($RMV/P_{ET}CO_2$ $lpm \cdot torr^{-1}$) for each P_IO_2 tested. The results from 4 incomplete experiments are not included.

VENTILATORY RESPONSE SLOPES

SUBJECT	0.21 ATA P_{IO_2}	1.75 ATA P_{IO_2}	2.80 ATA P_{IO_2}
DV	2.4883	6.0680	2.1884
TA	1.7766	0.9586	0.7919
DV	2.1512	3.9966	3.6577
TA	1.2298	2.0675	1.1171
CM	2.5773	1.5313	0.9132
DV	2.4899	4.4700	3.7258
CM	2.4905	2.7030	1.3036
OD	2.8600	2.7774	1.9752
CM	4.4777	3.1978	2.2476
OD	2.2004	2.4280	1.3199
CP	2.9800	2.9345	1.8101
DS	2.2534	2.0886	2.0780
DS	1.7226	3.2358	3.3843
OD	1.9952	3.1283	1.5139
CM	3.1861	2.8845	2.2408
OD	1.3701	2.2074	1.6045
CM	1.9089	2.8962	2.1694
TA	1.5213	1.8301	1.2110
AE	0.9318	2.3794	0.6595
CM	2.0712	2.4171	2.2326
AE	1.6350	2.8450	1.7114
SH	1.1370	0.8516	0.8027
AE	1.0736	2.9469	1.5332
CM	2.8594	3.0968	2.8135
CM	2.4977	1.9560	1.6287
OD	0.6816	1.1609	1.3443
DS	2.6117	2.5386	2.9580
AE	1.2548	1.9662	1.2024
CP	2.6948	2.8594	1.4881
TS	2.1267	2.1362	2.5970

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