Federal Aviation Administration

DOT/FAA/AM-13/22
Office of Aerospace Medicine
Washington, DC 20591

Physiological Determinants of Human Acute Hypoxia Tolerance

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November 2013

Final Report

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Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-13/22		

4. Title and Subtitle	5. Report Date
Physiological Determinants of Human Acute Hypoxia Tolerance	November 2013
	6. Performing Organization Code
7. Author(s)	8. Performing Organization Report No.
Self DA, Mandella JG, White VL, Burian D	
9. Performing Organization Name and Address	10. Work Unit No. (TRAIS)
FAA Civil Aerospace Medical Institute P.O. Box 25082 Oklahoma City, OK 73125	11. Contract or Grant No.
12. Sponsoring Agency name and Address	13. Type of Report and Period Covered
Office of Aerospace Medicine Federal Aviation Administration 800 Independence Ave., S.W. Washington, DC 20591	14. Sponsoring Agency Code

15. Supplemental Notes

Work was accomplished under approved task AM-11001

16. Abstract

Introduction. We investigated possible physiological determinants of variability in hypoxia tolerance in subjects given a 5-minute normobaric exposure to 25,000 ft equivalent. Physiological tolerance to hypoxia was defined as the magnitude of decline in hemoglobin saturation (SpO_2 - dependent variable).

Methods. Pulmonary function, heart rate variability (HRV), total hemoglobin, a $\dot{V}O_2$max estimation and resting oxygen consumption ($\dot{V}O_2$) were measured prior to the normobaric hypoxia exposure. Cerebral oximetry, ECG, middle cerebral artery blood flow velocity, noninvasive beat-to-beat arterial pressure and its first derivative, cardiac output, and left ventricular stroke volume, cerebral pulse oximetry, and hemoglobin oxygen saturation were recorded. Additionally, tidal volume and respiratory rate, breath-by-breath inhalation and end-tidal O_2, CO_2, and N_2 tensions were measured and $\dot{V}O_2$ computed. Mixed venous PO_2 and alveolar-capillary O_2 gradient was calculated. Serum S100b, a putative marker for cerebral hypoxic insult, was also measured in 26 subjects.

Results. Multivariate linear regression analysis was used to evaluate the ability of combinations of physiological measures to predict declines in SpO_2 in 34 subjects. Seven variables were identified that gave a statistically significant prediction model that accounted for 71% of the variance (R^2 = .706; adjusted R^2=.627).

Discussion. The model predicted that subjects with large total lung diffusion capacities for O_2, those with the highest end- alveolar PO_2 and the lowest mixed venous PO_2 at the end of the 5-min exposure, and those who maintained an O_2 consumption rate that exceeded their resting levels had the smallest declines in SpO_2. Additionally, cerebral oximetry declines were negatively correlated with SpO_2 declines and suggest that greater O_2 extraction at the tissue level may be a strategy for lowering oxygen tension in blood returning to the lungs, thus providing a larger gradient for diffusion.

17. Key Words	18. Distribution Statement
Hypoxia, Time of Useful Consciousness, Physiological Predictors, Normobaric Hypoxia, Hypobaric Hypoxia	Document is available to the public through the Internet: www.faa.gov/go/oamtechreports/

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	16	

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

ACKNOWLEDGMENTS

The authors thank the Airman Education Division at CAMI for their invaluable assistance in conducting the altitude chamber flights. We also thank Lawrence Paskoff, for his help in conducting this research and in manuscript preparation; and Nick Webster, MD and James Whinnery, Ph.D, MD for providing Medical Monitor coverage during the hypoxia exposures. Finally, we express our gratitude to the volunteer subjects who participated in this study.

Physiological Determinants of Human Acute Hypoxia Tolerance

CONTENTS

INTRODUCTION

The purpose of this research was to investigate the causes of variability in hypoxia tolerance using a healthy population and to investigate the usefulness of a protein marker in grading the severity of hypoxic insult to the brain.

Altitude chamber training has been the traditional method of demonstrating the subjective effects of acute hypoxia to aviators. Many military aeromedical training centers, as well as the Federal Aviation Administration (FAA), utilize a 5-min hypobaric exposure to a 25,000 ft equivalent environment to conduct this training. There is common experience among altitude chamber inside observers that the time to incapacitation is highly variable. Early work (Hoffman, Clark & Brown, 1946) documented the substantial variability in declines in oxygen saturation during exposures to simulated 28,000-38,000 ft altitudes among humans.

This variability has led to discussions between medical members of the National Transportation Safety Board (NTSB) and the FAA about whether to continue to include information relating times of useful consciousness (TUC), a measure of hypoxia tolerance to increasing altitudes (Table I) in the FAA Advisory Circular 61-107B (U.S. Department of Transportation, 2013).

Table I. AC 76-107B table comparing time of useful consciousness (TUC) following slow and rapid decompressions to the altitude at which they occurred. As altitude increases, the TUCs decrease. A rapid decompression cuts the TUC in half because the gasses in the respiratory tract undergo expansion in accordance with Boyle's Law, resulting in the loss of dead space air, which had the highest oxygen content.

ALTITUDE (ft)	TUC	Following Rapid Decompression
18,000	20 - 30 min	10 - 15 min
22,000	10 min	5 - 6 min
25,000	3 - 5 min	1.5 - 2.5 min
28,000	2.5 - 3 min	1 - 1.5 min
30,000	1 - 2 min	30sec – 1 min
35,000	30 sec - 1 min	15 - 30 sec
40,000	15 - 20 sec	Nominal
43,000	9 - 12 sec	Nominal
50,000	9 - 12 sec	Nominal

There is concern that the table may be misleading to the reader because of the considerable ranges in TUCs given. For instance, at 25,000 ft, TUC has a full 2-min range. TUCs are based on data that represent average values and reflect wide variation among individuals in terms of time to incapacitation. Rather than delete the table from the Circular, we have sought to augment it with more specific information on why hypoxia tolerances vary among individuals.

Earlier aviation-related quantitative work investigated the physiological basis for acute-onset hypoxia tolerance (Ernsting, 1963; Lilienthal, Riley, Proemmel, & Franke, 1946) but not the causes of variation among individuals, *per se*. In this study, we tested the hypothesis that differences in hypoxia tolerance among individuals can be accounted for by individual and clusters of measurable physiological variables. We chose variables that have been historically associated with both anaerobic and aerobic metabolism, as well as with responses to acute onset hypoxia.

We have defined physiological tolerance to hypoxia as the magnitude of hemoglobin desaturation (dependent variable). However, in order to connect hemoglobin oxygen saturation (SpO_2) declines directly to brain function and indirectly to TUCs, we also tested our subjects for the appearance of a brain-specific harbinger of anoxic insult. The protein S100b is a putative marker for hypoxic brain injury. S100b is strongly expressed in most neural tissues and at low levels in white blood cells (CD8+ cytotoxic T cells) and is found in serum at levels typically less than 30 pg/mL. It has been widely hypothesized that S100b enters the cardiovascular system due to disruption of the blood-brain barrier during hypoxia (BBB; Andersson, Linér & Henrik, 2009; Beharier, Kahn, Shusterman & Sheiner, 2012; Siman et al., 2009).

MATERIALS AND METHODS

Subjects

A convenience sample of volunteer research subjects that met the requirements for altitude chamber training was recruited from the pool of registrants for FAA physiological training classes. Students wishing to volunteer to participate in the study were asked to self-identify during the first hour of the class. Each subject provided written informed consent before participating, completed a health questionnaire, and possessed a current Class III Airman Medical Certificate. Measures from 34 healthy subjects (29 men and 5 women) not acclimated to high altitude comprised the dataset. The study protocol was approved in advance by the Civil Aerospace Medical Institute (CAMI) Institutional Review Board for the Protection of Human Subjects, which conforms to the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects. Subjects were given both a hypobaric (as part of the Physiological Training class) and normobaric (experimental condition) exposure to reduced oxygen tension (PO_2) environments.

Procedure

All subjects were given their hypobaric exposure in the morning and their normobaric exposure in the afternoon of the same day. The chamber flight was the training profile flight used by the FAA during the physiological training course described previously (Self, Mandella, Prinzo, Forster & Shaffstall, 2011).

Normobaric exposures were accomplished using a reduced oxygen breathing device (ROBD, Environics, Inc, Tolland, CT). The ROBD uses thermal mass flow controllers to mix breathing air and nitrogen to produce the sea level equivalent atmospheric oxygen contents for altitudes up to 35,000 ft. The device is calibrated on a primary flow standard traceable to the National Institute of Standards and Technology (NIST). The percentage of oxygen the device delivered was adjusted to account for the differential effects of water vapor pressure on alveolar gas composition at different $P_{b,}$ using the technique of Conkin (2011). To simulate the inspired fraction of O_2 (FIO_2) seen at a barometric pressure corresponding to 25,000 ft, we set the ROBD to deliver a PO_2 of 52 mm Hg in the inhaled gas mixture.

The Normobaric Breathing Apparatus (Figure 1)

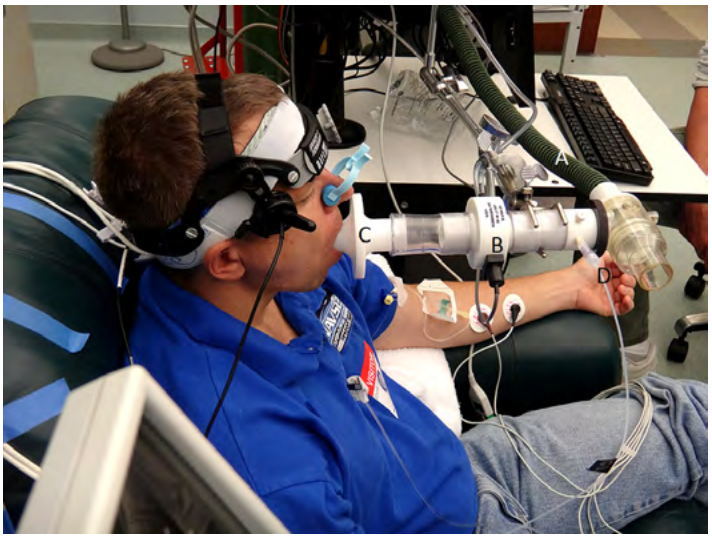

Figure 1. Photograph of the experimental setup showing the normobaric breathing apparatus. See text for description.

The ROBD outlet hose (A) was connected to the inlet port on a two-way non-rebreathing valve (Hans Rudolph, Inc, Shawnee, KS) having three ports. The inhalation and exhalation ports each had a unidirectional diaphragm that directed flow to and from the mouth. This valve was connected in series with a heated (37°C), low dead space pneumotachograph (B) that had a linear voltage output from 0 to 800 l/min (model 3813A series; Hans Rudolf, Shawnee, KS; dead space, 87.8 ml; flow resistance, 0.3 to 8.2 cmH2O/80-800L/ min). A spirometry microbial/ moisture filter mouthpiece (Care Fusion, Inc, Yorba Linda, CA, dead space: 50 ml) was attached to the pneumotachometer (C). Total dead space in the apparatus was 248.5 ml. A sampling line (D) connected to a mass spectrometer (model MGA-110; Perkin-Elmer, Waltham, MA) from a port close to the non-rebreathing valve, monitored the composition of inhaled and exhaled gasses.

As an orientation, all subjects breathed ground-level ambient air through the circuit for 2 min prior to the altitude exposure. That the apparatus had negligible physiological effects was evidenced by lack of changes in oxygen saturation or end-tidal CO_2 concentrations.

The normobaric exposure commenced by the subject simply switching from breathing room air to the 25,000- ft equivalent gas mixture supplied by the ROBD. Subjects were instructed to breathe normally, and we made no attempt to control end-tidal carbon dioxide tension (PCO_2).

Physiological Measures

Measurement of inhaled atmospheric and end-tidal air composition

A Perkin-Elmer Medical Gas Analyzer MGA-1100 (PerkinElmer Life and Analytical Sciences, Inc. Waltham, MA) was utilized for real-time measurement of the percentages of atmospheric gases (N_2, O_2, CO_2, and H_2O) delivered by the ROBD, and in the subjects' exhaled end-tidal gasses. Voltage output linearity was 0.5% of full scale for all gasses. Two-point calibrations were performed before each experiment using room air and a certified calibration gas (3% CO_2, 0% O_2, 97% N_2; ± 1%).

Total Lung Diffusion Capacity for Carbon Monoxide (DLCO)

DLCO was measured with the single breath-constant expiratory flow technique and calculated according to the American Thoracic Society's 1995 update and ATS-ERS 2005 guidelines (American Thoracic Society, 1995). DLCO was measured in each subject in the morning 2 hr prior to the chamber flight using the VMax 229 Encore Respiratory Diagnostics System (Care Fusion, Inc., Yorba Linda, CA). All the measurements were conducted with the subjects in sitting posture using a mouthpiece (dead space = 50 ml) and a nose clip. The system was calibrated at the source to read all measures at body temperature and pressure saturated with vapor (BTPS). The system calculated DLCO using standard Jones-Meade criteria (Jones & Meade, 1961) and an anatomic dead space algorithm (Hart, Orzalesi & Cook, 1963). Diffusing lung capacity for CO was determined twice with washout intervals of 4 min (the average was taken as the final result). The maneuver was performed using a test gas with 0.3% CO-0.3% methane-21% O_2-balance N_2. DLCO measurements were adjusted for the subject's own total hemoglobin value and then converted to DLO_2 using the method of Comroe et al. (Comroe, Forster, Dubois, Briscoe & Carlsen, 1962). The system was calibrated before each experiment by using a certified 3-liter calibration syringe. Calibration was achieved when measured stroke volume was within ± 3% of syringe volume.

Ventilation

Ventilatory flow and respiratory rate were measured with a heated, low dead space pneumotachograph (previously described) connected to a differential pressure spirometer (AD Instruments, Colorado Springs, CO) and acquired at a sampling rate of 1KHz on a personal computer for subsequent analysis.

Tidal volume was calculated on a breath-to-breath basis after digital integration of the flow signal at actual barometric pressure using LabChart Pro software V.7 (AD Instruments, Colorado Springs, CO). The system was calibrated before each experiment as previously described.

$\dot{V}O_2$max Estimation

Each subject was given a 6-min, sub-maximal Åstrand Bike Test (Åstrand & Ryhming, 1954; Buono, Roby, Micale & Sallis, 1989; Legge & Banister, 1986) to provide an estimate of $\dot{V}O_2$max. The test was conducted on a Velotron Dynafit Pro computer-controlled, precision electronic bicycle ergometer/trainer (Racermate, Inc, Seattle. WA). The energy output in watts of the rider was monitored with proprietary software (Racermate, Seattle, WA) that had a self-calibrating function. Heart rate was recorded continuously and was averaged during the last minute. $\dot{V}O_2$max was estimated using the formulae

$$\text{Females: } \dot{V}O_2\text{max} = \frac{(0.00193 * workload + 0.326)}{(0.769 * HR - 56.1)} * 100$$

$$\text{Males: } \dot{V}O_2\text{max} = \frac{(0.00212 * workload + 0.299)}{(0.769 * HR - 48.5)} * 100$$

upon which the Åstrand & Rhyming nomograms are based (Buono et al., 1989; Glassford, Baycroft, Sedgwick & MacNab, 1965). The correlation of the Åstrand Bike Test to actual $\dot{V}O_2$max has been shown to be 0.85-0.90 (7). This test was performed in the morning, 2 hr prior to the chamber flight.

Resting Oxygen Consumption

Resting oxygen consumption ($\dot{V}O_2$) was measured immediately prior to the normobaric hypoxia exposure. Breath-by-breath respiratory gas exchange was measured with the VMax 229 Encore Respiratory Diagnostics System (Care Fusion, Inc., Yorba Linda, CA), using a flow-through mask. A mass-flow sensor measured volume and airflow, and it was calibrated before each measurement by using a certified 3-L calibration syringe. Calibration was described previously. The system analyzed expired gas for oxygen concentration using a paramagnetic oxygen analyzer and for carbon dioxide concentration by using a nondispersive infrared analyzer. Gas analyzers were calibrated before each measurement using three known standard gas concentrations (16% O_2, 4% CO_2; 26% O_2, 0% CO_2; room air 20.94% O_2, 0.05% CO_2). Calibration was complete when gas analyzers measured oxygen and carbon dioxide concentration within ±5% of expected. Measurement was considered complete once a 5-min steady-state period was achieved or after 30 min, whichever occurred first. $\dot{V}O_2$ values were recorded every minute, and then averaged over the entire epoch. The VMax 229 was connected to an IBM-compatible personal computer for management and storage of data by using the CardioSoft/VMax Vision software for Windows (version 6.51, Care Fusion, Inc., Yorba Linda, CA). In practice, the subjects were tested in the same sitting position as the subsequent hypoxia exposure.

Oxygen Consumption at the End of the Hypoxia Exposure

Oxygen consumption ($\dot{V}O_2$) was measured at the end of the 5-min normobaric hypoxia exposure. Inhaled and end-tidal O_2, CO_2, and N_2 percentages, inhalation volume (V_{in}) converted to standard temperature pressure dry (STPD) volumes, and respiratory rate were averaged over the final 5 breaths of subjects before recovering on 100% O_2. V_{in} was adjusted for both anatomic and spirometry apparatus dead space. Anatomic dead space was estimated in each subject as a function of height using the relation

$$V_d = [7.585*(\text{height}^{2.363})]*10^{-4}$$

established by Hart et al. (1963; r=.917)). $\dot{V}O_2$ was then computed using the Haldane transformation (Haldane, 1912 & 1922) of the Fick principle through the following equation:

$$VO_2 = \left(V_{in} * \%O_{2in}\right) - \left[\left(\frac{\%N_{2in}}{\%N_{2\,\text{exp}}} * V_{in}\right) * \%O_{2\,\text{exp}}\right] * bpm$$

Measurement of Total Hemoglobin (SpHb)

Subjects were assessed for SpHb prior to the chamber flight and then again in the afternoon prior to the normobaric exposure. A pulse co-oximeter (Masimo Rainbow SET®, Masimo, Irvine, CA) with a spectrophotometric sensor (rainbow® DCI®) with multiple wavelengths of light was used to measure SpHb, along with oxygen saturation and pulse rate. The Pulse CO-Oximetry method discerns the distinctive light-absorption characteristics of different hemoglobin species and applies proprietary algorithms to determine total hemoglobin measurement.

Hemoglobin Oxygen Saturation

We monitored SpO_2 continuously throughout both the altitude chamber and ground level exposures. Percent hemoglobin saturation, expressed as the ratio of oxyhemoglobin to reduced hemoglobin in arterial blood, was measured with a pulse oximeter clamped to the index finger of subjects' non-dominant hand and with a forehead sensor placed above the right supraorbital ridge. Both emitted light at 660 and 940 nm. Absorption ratios and heart rate (HR) were then computed by a pulse oximeter (Nellcor Model N600-X, Covidien Corp, St. Louis, MO) and displayed as percent saturation and beats per minute (bpm), respectively. Continuous estimated arterial PO_2 was derived directly from SpO_2 measurements, using an inverse solution to the Severinghaus equation (Ellis, 1989).

Cerebral Tissue Regional Oxygen Saturation

Changes in regional brain blood oxygen status (rSO_2) were continuously monitored during the hypoxia exposure and expressed in relative units with functional near-infrared spectroscopy (Somanetics Corp., Troy, MI). This device measured changes in oxygenation, influenced by delivery and consumption in each cerebral hemisphere simultaneously. Regional brain oxygen saturation measured by this device reflected the balance between cerebral oxygen supply and demand in a mixed vascular bed dominated by gas exchanging vessels (Edmonds, Ganzel & Austin, 2004).

Finger Plethysmographic Hemodynamic Variables

Beat-to-beat arterial blood pressure (ABP) and its first derivative were continuously recorded using finger photoplesthysmography (NexFin, BMEYE B.V., Amsterdam, The Netherlands). ABP values were corrected to heart level using the NexFin height correction system. In practice, a finger cuff was attached to the middle phalanx of the right third finger to measure finger arterial blood pressure. Stroke volume was determined by a three-element model of arterial input impedance (Nichols & O'Rourke, 1990). Cardiac output was calculated as the product of stroke volume and heart rate. Manual blood pressure measurements were taken before and after testing, and from these, a DC offset was computed and applied *post hoc* to align the plethysmography-derived values with directly measured pressures.

Intracranial Blood Flow

Continuous mean, systolic, and diastolic blood flow velocity in the right and left middle cerebral arteries (MCAs) were measured in all subjects. Backscattered Doppler signals, available as analog voltages, were continuously and simultaneously monitored by means of a ST3 pulsed digital transcranial Doppler (TCD) system (model # PMD 150, Spencer Technologies, Seattle, WA). Ipsilateral MCA velocity measurements were made using 2- MHZ probes via the posterior temporal windows immediately above the zygomatic arch. Middle cerebral arteries were identified bilaterally within depths of isonation between 35 to 56 mm. Constant position of the ultrasound probes was ensured by use of a head frame probe holder. After individual adjustment of Doppler variables (gain, sample volume, and power of ultrasound), these were not changed over the period of the hypoxia exposure.

Electrocardiogram (ECG) and Heart Rate Variability (HRV) Studies

ECG recordings were obtained with the subjects in a semi-recumbent position in a dark, quiet room for 5 min after an equilibration period of 5 min using a modular bioamp (Powerlab Model ML880, AD Instruments, Bella Vista, Australia). This duration of recording gave a good definition of frequency domain measures and acceptable resolution of time domain measures ("Task Force," 1996). ECG data were analyzed off-line using the HRV Module 1.01 for Chart 5 (AD Instruments, Bella Vista, Australia). All ECGs were digitally recorded at 1 KHz according to the standards of measurements, physiological interpretation, and clinical use guidelines for assessment of HRV ("Task Force," 1996). Time and frequency domain measures were calculated for HRV. Frequency domain parameters of HRV were derived using power spectrum analysis (fast Fourier transforms) with high-frequency power (HFP), defined as 0.15–0.40 Hz; and low-frequency power (LFP), defined as 0.04–0.15 Hz and expressed in normalized units, adjusting for changes in total power. The ratio of LFP/HFP was the only HRV measure considered as a predictor of hypoxia tolerance.

Estimation of Mixed Venous PO_2

To examine the gradient for oxygen diffusion, mixed venous PO_2 ($MVPO_2$) averaged over the last 5 breaths of the hypoxia exposure was estimated through the use of the Johns Hopkins School of medicine gas exchange lung model (Ball, Nehal & Rosner, 2013). The integrations used by the model (described previously by Michaelson, Sackner & Johnson, 1973) are derived from the fundamental equation:

$$DLO_2 = \frac{\dot{V}O_2}{PAO_2 - MVPO_2}$$

The entering arguments for the model were: cardiac output, inspiratory tidal volume, respiratory rate, respiratory quotient (from resting $\dot{V}O_2$), anatomic dead space, P_b, measured PAO_2, total hemoglobin, measured total lung diffusion capacity for oxygen, and $\dot{V}O_2$. We then subtracted calculated $MVPO_2$ from PAO_2 to determine the alveolar-to-pulmonary capillary gradient over the last 5 breaths of the hypoxia exposure.

Serum S100b Measurement

Whole blood samples were collected in Serum Separator Tubes (SST; BD Vacutainer® SST™ 367986; BD, Franklin Lakes, NJ) at four times over a 5.5-hr span. The first sample (T_0) was collected at 9:00 AM before the chamber flight. A second sample (T_1) was collected at 2:00 PM, approximately 2 hr after the 5-min hypobaric (25,000 ft) exposure and immediately preceding the 5-min normobaric 25,000-ft exposure. The third sample (T_2) was collected immediately following the normobaric exposure and a final sample (T_3) collected 15 min later. After at least 30 min, SSTs were centrifuged at 1300*g for 10 min at 10° C in a swinging bucket centrifuge. Serum was aliqoted into 500uL aliquots and stored at -80°C until assayed.

S100b concentrations were determined in triplicate from 100 uL of serum by enzyme-linked immunosorbent assay (ELISA) kits (P/N KA0037; Abnova Corp, Jhongli City, 320 Taiwan) according to the manufacturer's protocol except that signal detection was achieved with the Sword Peroxidase Assay (Sword Diagnostics, Chicago, Ill). Due to the added sensitivity of the Sword Assay, standard curves were made per the Abnova protocol with further serial dilution to 6.25pg/mL. A mixed-effects longitudinal model was applied to the serum S100b and physiological data. S100b expression data alone was analyzed with a random-effects model to determine differences in serum S100b levels compared to the baseline sample collected at 9:00 AM.

Data Acquisition and Recording Scheme

Analog signals from all except ventilatory and ECG sensors were digitized at 20 samples/second and recorded with a custom-built LabView data acquisition instrument (National Instruments Corp., Austin, TX). Ventilatory signals (flow, volume, and rate) and ECG were digitized at 1 KHz and recorded using a modular Bio Amp (Powerlab Model ML880, AD Instruments, Bella Vista, Australia). Data files were synchronized *post hoc* by introducing a generated square wave with a function generator (Hewlett Packard model 3310B; Palo Alto, CA) into both data acquisition systems during the experiment.

Analysis

Statistical analysis was performed using programs available in the SPSS statistical package (SPSS, version 15.0, Chicago, IL). Significance was set *a priori* at alpha ≤ 0.05. Measurements are presented as means ± SD. All variables were tested for normal data distribution. Bivariate analysis between predictor variables and the magnitude of SpO_2 declines was carried out using Pearson product-moment correlation coefficients. Multivariate regression analysis of the fall in arterial SpO_2 over the 5-min exposure to a 25,000 ft equivalent environment (dependent variable) against possible physiological predictors of hypoxia tolerance was performed to look for independent associations. Squared values of the predictors identified in the bivariate analyses were used to construct a linear model that accounted for variance in the dependent variable. Once the regression models were obtained, standardized regression coefficients were calculated. This enabled the comparison of the relative contribution of individual predictors to the R^2 while controlling for the units of measurement and range of values for each predictor. The value of the standardized regression coefficients represent the change of the response variable, expressed in units of its SD, when the predictor variable changes by 1 SD. Adjusted R^2 values (R^2 corrected for the number of observations and the number of predictors in the analysis) was also computed.

RESULTS

Characteristics for the 34 subjects (29 men and 5 women) included in the data set are presented in Table II. Bivariate comparisons between characteristics yielded one significant relationship: $\dot{V}O_2max$ and DLO_2 (r = 0.524, p= 0.001).

The physiological parameters we measured during the normobaric hypoxia exposure, along with their descriptive statistics, are presented in Table III. Measures acquired during the 5-min exposure are expressed as changes from baseline values at the start of the exposure, excepting for SpO_2, PAO_2, and computed $MVPO_2$. Differences in hemoglobin saturation and alveolar and computed mixed venous oxygen tension just prior to the hypoxia exposure did not significantly differ among subjects and are reported as absolute values.

Table II. Anthropometric and physiological characteristics of 34 subjects. Values reported are means ± SD along with ranges.

Age (Years)	37.50 ± 12.38 (19 – 64)
Height (cm)	175.95 ± 8.63 (161.29 – 191.77)
Weight (kg)	81.29 ± 15.20 (58.18 – 130.91)
Estimated VO_{2Max} (ml/min/kg)	45.72 ± 10.40 (25.30 – 70.43)
Resting VO_2 (ml/kg/min)	3.93 ± 0.90 (2.76 – 6.35)
DLO_2 (ml/mm Hg/min)	40.65 ± 7.72 (25.83 – 59.00)
Total Hgb (g/dL)	14.55 ± 1.06 (12.45 – 16.10)
HR variability (LF/HF)	2.67 ± 3.37 (0.20 – 15.80)

Table III. Responses to the normobaric exposure to 25,000 ft equivalent (PO_2=52 mm Hg). Values reported are means ± SD along with ranges. End $\dot{V}O_2$ – Resting $\dot{V}O_2$, PAO_2, and $MVPO_2$ are expressed as actual values at the end of the exposure. All other measures are expressed as change from values measured just prior to the start of exposure to the hypoxic gas mixture.

End $\dot{V}O_2$ – Resting $\dot{V}O_2$ (ml/kg/min)	-0.68 ± 1.03 (-2.39 – 1.84)
End SpO_2 finger (% saturation)	52.61 ± 9.67 (33.70 – 79.90)
End O_2 Sat Head (% saturation)	67.08 ± 7.05 (48.40 – 82.20)
Change in cardiac output (L/min)	3.60 ± 1.94 (0.45 – 10.58)
Change in stroke volume (ml)	-4.41 ± 14.79 (-32.30 – 43.00)
Change in tidal volume (L)	.15 ± 0.46 (-0.98 – 1.33)
Change in respiratory rate (breaths/min)	4.25 ± 4.60 (-3.18 – 17.40)
Change in HR (beats/min)	36.25 ± 18.92 (-24.40 – 104.00)
Change in MAP (mm Hg)	7.08 ± 11.30 (-22.90 – 31.33)
Change in middle cerebral artery blood flow velocity (cm/sec)	19.68 ± 12.02 (-14.40 – 45.00)
End PAO_2 (mm Hg)	34.35 ± 3.03 (37.96 – 41.80)
End $MVPO_2$ (mm Hg)	26.37 ± 2.37 (22.90 – 31.00)
End PAO_2-MVO_2 gradient (mm Hg)	7.98 ± 3.65 (1.60 – 16.90)
Decline in $PACO_2$ (mm Hg)	9.30 ± 3.37 (2.63 – 14.30)
Change in rSO_2 (relative units)	27.27 ± 4.95 (17.30 – 36.00)
Change in arterial pressure dPdt (mm Hg/sec)	471.78 ± 273.07 (-238.00 – 927.00)

Forehead SpO_2 and HR showed very similar frequency of response characteristics in all subjects, with finger SpO_2 (dependent variable) always exhibiting slower response times in both desaturation and in resaturation when 100% O_2 was breathed at the end of the 5-min period (Figure 2). Both SpO_2 and rSO_2 recordings always showed a biphasic desaturation response and were negatively correlated (r= -0.384; p= 0.025). An initial rapid decline was seen that roughly corresponded to one complete circulation time. This was followed by a slower desaturation rate, sometimes interspersed with brief plateau periods lasting several seconds.

ABP tended to increase with exposure to hypoxia, but the magnitude was variable across subjects. Finger plethysmography-derived CO always increased during the exposures, but this was due almost entirely to HR changes, with SV typically declining, reflecting decreased diastolic filling times. The first derivative of peak systolic pressure always increased during the hypoxia exposure. HR showed increases as SpO_2 and rSO_2 declined in all subjects. The magnitude of changes in HR was negatively correlated with the HRV measure LF/HF (r= - 0.369; p= 0.032).

End tidal O_2 and CO_2 tensions fell during the hypoxia exposure but were not significantly correlated. $PACO_2$ was correlated with respiratory rate (r= 0.382, p=.026) but not tidal volume. Respiratory rate consistently changed to a greater extent than tidal volume in response to hypoxia. DLO_2 and changes in respiratory rate were negatively correlated (r = -0.486, p= 0.004), and changes in V_t and O_2 consumption-use deficit showed a positive correlation(r = 0.353, p= 0.040). Computed $MVPO_2$ fell during the hypoxia exposures and was not correlated with SpO_2 declines. However, the PAO_2-$MVPO_2$ gradient for oxygen diffusion was significantly correlated with SpO_2 declines (r= 0.435; p= 0.010): the larger the gradient, the less SpO_2 fell during the 5-min exposure.

Max velocity of MCA flow gradually increased after the start of the hypoxia exposure in all subjects and then abruptly decreased within seconds of inhalation of 100% O_2. There was asymmetry of the flow responses characterized by a slower onset than offset response (Figure 3). Changes in MCA flow velocity were not significantly correlated with changes in MAP, $PACO_2$, or HR. They were, however, negatively correlated with changes in PAO_2 (r= -0.367, p= 0.033).

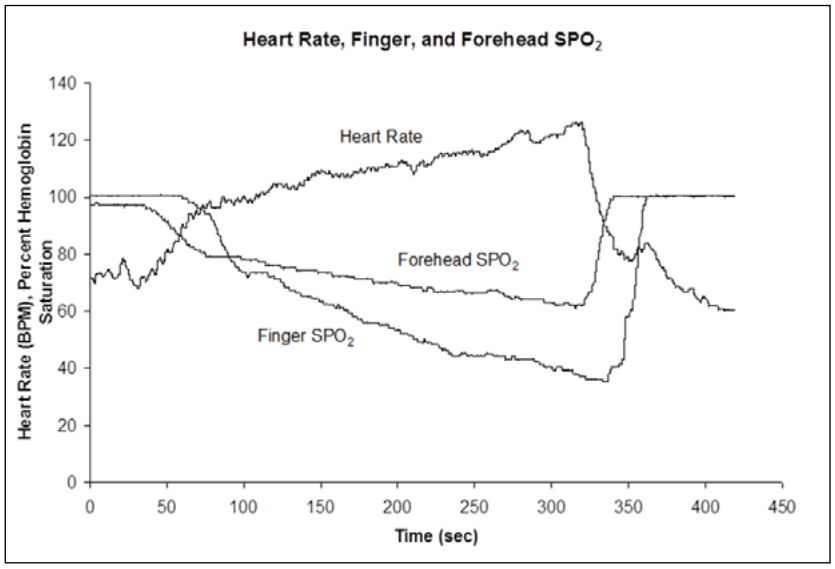

Figure 2. SPO2 and HR records for one subject showing the temporal relationships between the forehead and finger sensors, and their lags following changes in HR.

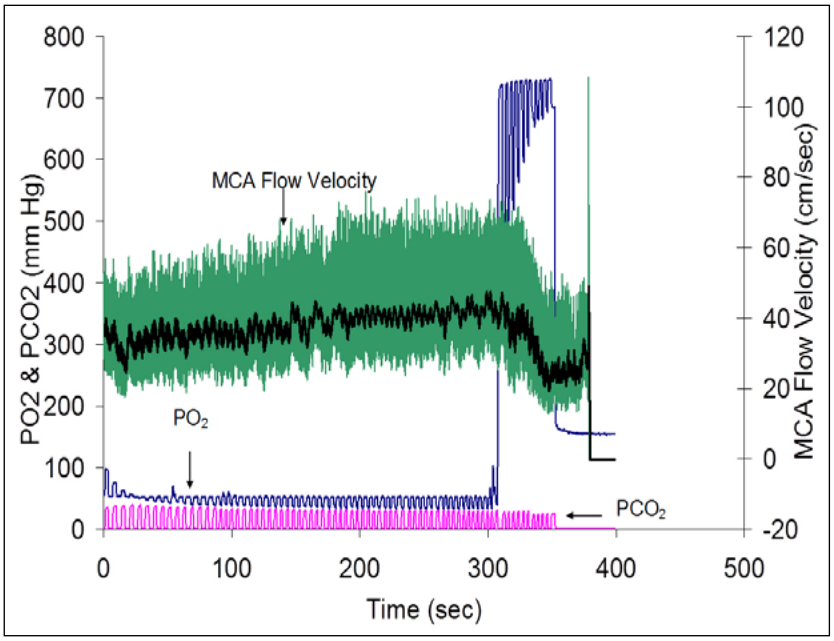

Figure 3. Beat-by-beat MCA flow velocity in one subject during a 5-min normobaric hypoxia exposure. Also shown are PO_2 (blue) and PCO_2 (pink). Breath-by-breath traces of respiratory gasses expressed as partial pressures (Pb*fractional component). For oxygen, the top part of each breath corresponds to the inhalation phase and was set at 52 mm Hg. For CO_2, the top part of each breath corresponds to the exhalation phase. The spike in PO_2 at the end of the recording resulted from the subject inhaling 100% O_2 at the end of the 5-min hypoxia exposure. Moving average of the MCA flow velocity is overlaid. MCA velocity increased independent of arterial blood pressure during the course of the exposure and then rapidly declined as the subject inhaled 100% oxygen.

Average oxygen consumption over the last five breaths of each subject's hypoxia exposure was computed from the mass spectrometry and pneumotachometer signals (Figure 4). These values were then compared to the resting $\dot{V}O_2$ measurements made just prior to the hypoxia exposure. In general, subjects either consumed slightly more or slightly less than their resting $\dot{V}O_2$ (range: -2.39-1.84 ml O_2/kg/min; $\bar{X}$ = -0.68±1.03).

Regression Model

Multiple regression analysis was conducted to evaluate how well a set of physiological characteristics predicted tolerance to acute hypoxia. Physiological measures were used as predictor variables in a standard regression analysis to account for the variation in magnitude of the fall in SpO_2 (dependent variable), using the enter method. Preliminary analyses were performed to ensure no violation of the assumptions of linearity, normality, and homoscedasticity. Pearson product-moment correlation coefficients were used to indicate the magnitude and direction of relations among variables. From these, seven variables were selected that in combination, provided the strongest prediction of SpO_2 declines, yielding the equation

$SpO_2 = [-18.73 - (0.2\,\dot{V}o_2max) + (.383HRV) + (2.68\ End\ \dot{V}o_2 - Resting\ \dot{V}o_2) + (2.38PAO_2) + (.414DLO_2) - (.309\ PAO2\text{-}MVO_2\ gradient) - (.528rSO_2)]$

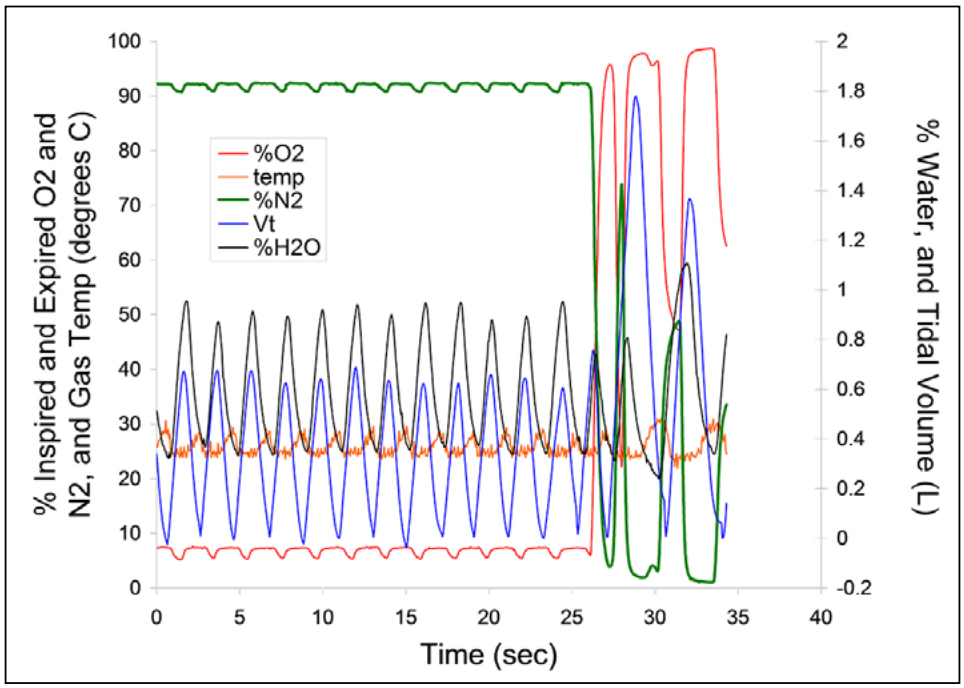

Figure 4. Ventilatory measures over the last 12 breaths taken during a hypoxia exposure in one subject. Shown are breath-by-breath tidal volume, % O_2, %N_2, %$H2_O$ and temperature. These parameters were used to compute average oxygen consumption averaged over the last 5 breaths taken by the subject during the 5-min hypoxia exposure.

The Pearson correlations (C) of the seven variables along with raw beta coefficients (*B*), their corresponding standard errors (SEB), standardized beta coefficients (B), *t* values and significance *p* values are shown in Table IV.

The prediction model was statistically significant: F (7, 26) = 8.94, $p < .0005$, and accounted for 71% of the variance of SpO_2 declines after a 5-min exposure to simulated 25,000 ft. ($R^2 = 0.706$, Adjusted $R^2 = 0.627$). Four predictor variables (end alveolar PAO_2, resting oxygen consumption – end O_2 uptake, DLO_2, and rSO_2) made statistically unique contributions to the regression model.

Table IV. Seven variables included in the multiple regression model that accounted for the variability in SPO2 declines (dependent variable). C = Pearson correlation, *B* = raw beta coefficient, SEB = standard error, B = standardized beta coefficient, *t* = t-test, *p* = significance. Note: The significance values show which predictors provided a unique contribution to the model (**), and are not associated with the Pearson correlations in column 1.

Model	C	*B*	SEB	B	*t*	*p*
Intercept		-18.73	20.4		-9.18	.367
$\dot{V}O_2$ MAX (ml/min/kg)	.027	-.200	.127	-.216	-1.57	.128
Heart Rate Variability (LF/HF)	.221	.383	.344	.133	1.11	.275
End O_2 use – Resting O_2 use (ml/kg/min)	.325	2.68	1.12	.286	2.39	.024**
End PAO_2 (mm Hg)	.671	2.38	.559	.689	4.25	.000**
Total Lung Diffusion Capacity for Oxygen (ml/mm Hg/min/min)	.224	.414	.158	.331	2.61	.015**
PAO_2-MVO_2 gradient (mm Hg)	.435	-.309	.446	-.117	-.692	.495
Change in rSO_2 units	-.384	-.528	.228	-.271	-2.32	.029**

Twenty-six study participants completed all four sample collections. Based on an assumption of low-level expression of S100b, a level of 5 pg/mL was assigned to samples below the assay level of quantitation. No physiological parameter was found to significantly explain differences in serum S100b levels by longitudinal modeling. Neither were serum levels correlated with the degree of SpO_2 declines. However, serum S100b levels were found to be statistically different between samples collected after the normobaric exposure (T2 and T3) and the baseline sample, T0 ($p<0.01$, Figure 5).

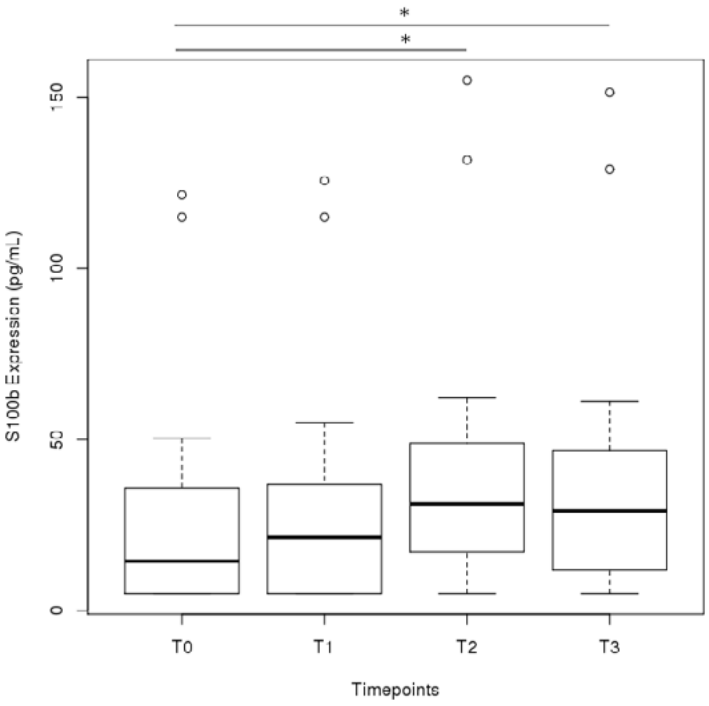

Figure 5. Boxplots of S100b expression values across the experimental time course. Boxes show the interquartile range of expression values within each time point. Asterisks (*) denote time point contrasts where $p<0.01$. Samples were drawn before the chamber flight (T0), 2 hrs after the chamber flight and just before the normobaric hypoxia exposure (T1), and immediately and 15 min after the normobaric exposure (T2 and T3, respectively).

DISCUSSION

We have investigated how well a group of physiological variables predicts the degree to which hemoglobin oxygen saturation falls when human subjects breathe a gas mixture with reduced oxygen tension. Previous work by Ernsting (1963) thoroughly examined cardiovascular, ventilatory, and respiratory gas composition effects of breathing pure nitrogen to unconsciousness. Our work builds on this foundation by adding additional detail to our understanding of the human response to hypoxia.

In our system, end tidal O_2 and CO_2 were allowed to vary freely as a function of each subject's response, with only inspired gas being controlled. Previous work has utilized gas mixing systems that "clamped" end tidal gasses at levels determined by the experimenter (Robbins, Swanson, Micco & Schubert, 1982). This technique provided a square or sinusoidal wave forcing function useful for studying individual end organ response characteristics. However, our work focused on organismal responses to simulated sudden high altitude exposure.

DLO_2 and total hemoglobin were included in the regression model. Although it was not a unique predictor of hypoxia tolerance, DLO_2 was correlated with $\dot{V}O_2max$. The strong correlation between $\dot{V}O_2max$ and DLO_2, but not between DLO_2 and SpO_2 declines during hypoxia exposure, suggests that hypoxia tolerance may be perfusion rather than diffusion-limited, at least for normobaric exposures to a 25,000-ft equivalent.

Numerous procedures have been validated to estimate $\dot{V}O_2max$ from submaximal exercise. We estimated it using the Åstrand Bike Test. This test, when compared against measured $\dot{V}O_2max$, has been shown to have correlation coefficients of 0.80 to 0.92 (Cengiz, Robergs & Kravitz, 2008; Glassford et al., 1965). In our study, estimated $\dot{V}O_2max$ scores were compared against the level of regular aerobic activity subjects described in the health questionnaire. In general, the test results reflected the rigor of the subjects' individual exercise regimens.

Changes in MCA flow velocity were not a predictor of SpO_2 declines and were negatively correlated with PAO_2 but not $PACO_2$ at the end of the 5-min hypoxia exposures. One explanation is that $PACO_2$-induced changes were dampened by changes in PAO_2, causing competing inputs to MCA vasomotor tone. MAP and ventilatory responses were not correlated with MCA flow velocity in this study. This may reflect both having not exhausted the cerebral autoregulatory responses to increased perfusion pressure, and only small reductions in end tidal PCO_2 in our subjects. Previous work showed that variability in acute hypoxic ventilatory responses increases in MAP, and MCA flow velocity were linked and were heavily influenced by end-tidal PCO_2 (Ainslie & Poulin, 2004). Our results may have been quite different at higher simulated altitudes where a more robust ventilatory response would have been induced.

The ratio of LF/HF in HRV studies has been frequently cited as a measure of autonomic balance, with HF components (0.15-0.40 Hz) representing vagal tone, and LF (0.04-0.25 Hz) components representing both sympathetic and parasympathetic elements ("Task Force," 1996). Including it as a predictor variable in the regression model increased the r^2. However, LF/HF was not a statistically unique contributor as the variability in SpO_2 declines, and bivariate analysis yielded an insignificant correlation. This would suggest that LF/HF exerts its effects indirectly through other predictors of SpO_2 declines.

Whether subjects had a deficit or surplus in oxygen consumption during the last 5 breaths of hypoxia exposure, compared to their resting $\dot{V}O_2$,was a unique predictor of hypoxia tolerance in our regression model. Furthermore, the alveolar-to-mixed venous PO_2 gradient was strongly correlated with SpO_2 declines and was included in the regression model. At any given PAO_2, $MVPO_2$ has considerable influence on the rate of diffusion of oxygen (Wagner, 1982). Previous work demonstrated that O_2 transport was defended during hypoxia by both increased ventilation and a reduction in mixed venous PO_2 (Sutton et al., 1988). In our subjects, the magnitude of the oxygen diffusion gradient predicted the fall in SpO_2: those with a higher gradient, and presumably a greater diffusion rate for oxygen, exhibited the smallest declines in SpO_2. rSO_2 was negatively correlated with falls in SpO_2, suggesting that increased tissue extraction of oxygen may have provided a mechanism for this.

In our calculation of $MVPO_2$, we used DLO_2 measured during normoxia. One possible source of error may have resulted from DLO_2 increasing during the hypoxia exposure due to capillary recruitment, effectively increasing the gas exchange surface area. However, earlier work (Lilienthal et al., 1946) demonstrated that DLO_2 increased relatively little during hypoxia at rest but increased dramatically during exercise.

We attempted to relate physiological tolerance to hypoxia, as measured by SpO_2 declines, to TUCs. Hoffman et al. (1946) related TUCs to SpO_2 declines and found that SpO_2 values averaged 64% at the appearance of the first error in performing a cognitive task, and 56% at the time of imminent unconsciousness. This relationship held regardless of the altitude, with the variation occurring only in the time to desaturation.

Our results appear to support a strong relationship between hypoxia as measured by SpO_2 declines and changes in serum S100b. This suggests that the level of hypoxic stress seen in our subjects was impacting brain physiology. We observed a statistically significant elevation of serum S100b after two 25,000-ft. equivalent altitude exposures. We were unable to show that the serum levels of S100b varied in a manner that reflected the magnitude of SpO_2 declines. Our results argue for a possible role of the protein as a reporter of brain hypoxia events but not their severity.

We conclude that tolerance to acute-onset hypoxia appears to be predictable through a group of physiological variables. Our model predicts that subjects with large total lung diffusion capacities for O_2, those with the highest end-alveolar PO_2, and the lowest computed mixed venous PO_2, and those who maintained an O_2 consumption rate that exceeded their resting levels had the smallest declines in SpO_2. Additionally, cerebral oximetry declines were negatively correlated with SpO_2 declines. This suggests that greater O_2 extraction at the tissue level may be a strategy for lowering oxygen tension in blood returning to the lungs, thus providing a larger gradient for diffusion.

Finally, these results have provided the FAA with information leading to a revision of the TUC table in the AC No: 61-107B. The following statement will be added under the TUC table and will, hopefully, lead to more informed decisions by aviators and improvements in flying safety.

CAUTION

TUC values are based on data that represent average values and reflect wide variation among individuals in time to incapacitation. This variation results from differences in an individual's total surface area for gas exchange in the lungs, total amount of hemoglobin available in the blood to bind oxygen, and oxygen consumption rate at rest (related to body mass index). Other sources of variation are the extent to which hypoxia stimulates increases in depth and rate of breathing, and increases in the amount of blood the heart pumps (faster heart rate). Finally, individuals able to increase the amount of oxygen they can extract from the blood in muscle and brain tissue are more hypoxia-tolerant. This is largely genetically determined but can be enhanced by physical conditioning resulting from a regular aerobic exercise program. As many of these factors will be unknown by the aviator, the safest approach would be to assume the lower TUC value is limiting.

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