Acute Hypoxic Hypoxia and Isocapnic Hypoxia Effects on Oculometric Features

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Introduction: Visual performance impairment after hypoxia is well recognized in military and civilian aviation. The aims of this study were: 1) to assess oculometric features such as blink metrics, pupillary dynamics, fixations, and saccades as cognitive indicators of early signs of hypoxia; and 2) to analyze the impact of different hypoxic conditions ["hypoxic hypoxia" (HH) and "isocapnic hypoxia" (IH)] on specified oculometrics during mental workloads. ***Methods:*** Oculometric data were collected on 25 subjects under 3 conditions: normoxia, HH (8% O_2 + balance N_2), and IH (7% O_2 + 5% CO_2 + balance N_2). The mental workload task consisted of reading aloud linear arrays of numbers after exposure to gas mixtures. ***Results:*** Blink rates were significantly increased under hypoxic conditions (by +100.7% in HH and by +92.8% in IH compared to normoxia). A faster recovery of blink rate was observed in transitioning from IH (23.6% vs. 76.3%) to normoxia. The percentage change in pupil size fluctuation was increased under HH more than under IH (29% vs. 4.4%). Under HH average fixation time and target area size were significantly higher than under IH. Total saccadic times under hypoxic conditions were significantly increased compared with normoxia. ***Conclusions:*** These results suggest that oculometric changes are indicators of hypoxia, which can be monitored using compact, portable, noninvasive eye-tracking devices in a cockpit analogous environment to detect hypoxia-induced physiological changes in aircrew. Comparative results between HH and IH support the potential role of carbon dioxide in augmenting cerebral perfusion and hence improved tissue oxygen delivery.

Keywords: acute hypoxia, oculometrics, capnic status, cognitive impairment, eye tracking.

THE EXPOSURE OF an organism to hypoxic stress activates an autonomic cascade with release of neurotransmitters that initiate physiological acclimatization mechanisms in response to the environmental challenge (23). During operations at high altitude, however, effects of cerebral hypoxia due to reduced ambient partial pressure of oxygen result in cognitive impairment that adversely influences judgment and decision-making capacity, thereby increasing the risk of operations (9). In the last few decades, research efforts have focused on noninvasive and neurophysiological biomarkers to detect early signs of hypoxia-induced performance changes reflecting central nervous system (CNS) impairment (21,26,28). Oculometric measures have been proposed as direct physiological signs of cognitive activity (12,19). Since vision has been recognized as being highly sensitive to hypoxia, oculometric measures such as pupillary dynamics, constrictor amplitude/latency, and saccadic velocity have been proposed as indicators of CNS hypoxia and altitude acclimatization (6,26,29). Cymerman et al. (6) reported that pupillary reflex and oculomotor performance metrics were significantly altered during hypoxia, manifesting as decreased pupil diameter, constrictor amplitude and latency, and an increase in saccadic velocity. The reduction in pupil diameter and constrictor amplitude have been associated with the known central depressive effects of hypoxia, while reduction in constrictor latency (i.e., faster reaction time) and increases in saccadic velocity have been attributed to the stimulatory effect of hypoxic sympathetic nervous system activation (6). Similar effects of hypobaric hypoxia on pupil size changes have been reported by Wilson et al. (29), who suggested pupillary dynamics as a direct measure of brain function. Thomson et al. (25) described pupillary diameter instability as due to fluctuation in activity of the sympathetic and parasympathetic innervations of the iris muscles when, during hypoxia, the level of consciousness or alertness is reduced. This might be the result of a direct, transient, and inhibitory effect of hypoxia on specific components of the CNS as well as an indirect stimulatory effect due to an increase in peripheral humoral factors. The autonomic response plays a significant role in the physiological adjustments to hypoxia, modulating vital functions such as blood pressure, stroke volume, and heart rate, all of which are increased in hypoxia (20).

While the concepts of changes in oculometrics have been observed in altitude environments, there are to date no studies that document early changes due to acute, severe exposure (> 6,096 m or 20,000 ft) to altitude. Moreover, few studies have analyzed the effect of different conditions of hypoxia (such as hypoxic hypoxia and isocapnic hypoxia) on these parameters. Gellhorn (8) first analyzed the role of carbon dioxide on

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cognitive performance, reporting that the negative effects of hypoxia on visual discrimination can be mitigated by adding carbon dioxide to the hypoxic gas mixture.

Therefore, we hypothesized that oculometric measures may represent candidate operational indicators in detecting early changes in different hypoxic conditions. Based on previous studies (8), we expected performance to improve when adding carbon dioxide to the hypoxic gas. The aims of this pilot study were: 1) to identify multiple oculometric measures (blink metrics, pupillary dynamics, fixations, and saccades) using a noninvasive and unobtrusive eye-tracking device during a validated challenge task (the King-Devick test) under aggressive acute hypoxic conditions; and 2) to analyze the impact of different acute hypoxic exposures (such as hypoxic hypoxia and isocapnic hypoxia) on specified oculometric measures and the potential specific effect of capnic status.

METHODS

Subjects

In this Mayo Clinic Institutional Review Board approved study protocol, 25 subjects (men:women, 14:11) were enrolled. Only recruits between 18 and 55 yr of age without significant cardiopulmonary and other disease (exclusion criteria were: history of obstructive/restrictive respiratory disease, cardiovascular disease, epilepsy, diabetes, chronic headaches or migraines, hematologic disorders) were enrolled. All subjects were nonsmokers and none were taking regular medications. A negative urine pregnancy test was required for female subjects. Informed consent was obtained from all subjects prior to enrollment. Subject demographics showed mean values of: age, 32.4 ± 9.8 yr; height, 1.74 ± 0.12 m; weight, 73.9 ± 16.3 kg; and BMI, 24.3 ± 4.1.

Equipment and Procedures

All studies were carried out in a quiet, climate-controlled room in the Aerospace Medicine and Vestibular Research Laboratory at Mayo Clinic Arizona (500 m or 1640 ft, ambient pressure 716 mmHg). The ambient light levels were controlled in all the experiments; the only relevant light source was the eye tracking screen, which was at stable luminance during the data collection. During the study, subjects were seated facing the Tobii T50 eye-tracking device (Tobii Technology, Stockholm, Sweden) to record eye movements. The eye tracking system consisted of a 17″ TFT display with infrared cameras which captured eye movements by tracking the reflections of infrared reference lights on the subjects' retinas. Data were collected at a rate of 50 Hz with an accuracy of 0.5°. To insure accurate tracking of eye movements, equipment calibration was performed for each subject prior to data collection. A mental workload task was created using a cognitive test [the King-Devick (K-D) test] (7) which was recently studied in a hypoxic environment (24). This test requires reading numbers aloud arranged from left to right on sequential lines shown on three different test cards (or screens). The K-D test presents an increasing level of difficulty with each successive card and also includes instructions to perform the reading as quickly as possible without error [for more details, refer to Galetta et al. (7)]. The K-D test cards were presented sequentially to each subject via the display monitor while detailed eye tracking data (**Fig. 1**) were collected simultaneously during each experiment.

A finger sensor for pulse oximetry recordings (Nonin Medical, Inc., Plymouth, MN) was placed on a distal phalanx of each subject. For the entire experiment, the subjects wore a Gentex military helmet and military tight fitting mask. The mask hose was occluded at the inlet and the subjects were asked to inhale, hence creating a vacuum to ascertain and ensure absence of leakage. The end-tidal CO_2 ($P_{ET}CO_2$) level was also recorded at the exhalation port of the aviator mask through a capnographic device (Nonin Medical, Inc., Plymouth, MN). Two different hypoxic conditions were induced by giving premixed gas mixtures through the mask. The

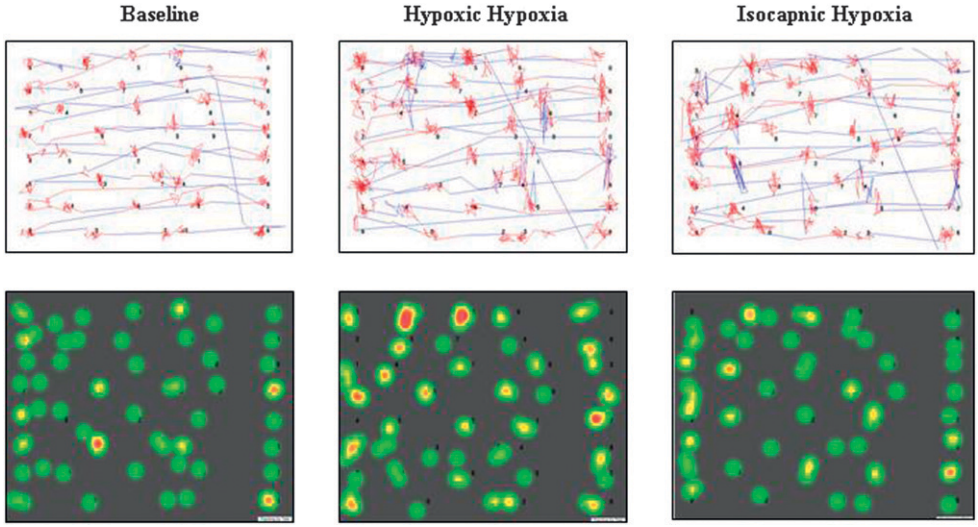

Fig. 1. Top panel: scan-paths consisting of saccades (in blue) and fixations (in red); bottom panel: fixations in the form of heat maps during the reading of sample K-D test card in the Baseline normoxic, hypoxic hypoxia (HH), and isocapnic hypoxia (IH) conditions.

hypoxic hypoxia condition (HH) was simulated by using an 8% oxygen (partial pressure 24.3 mmHg) with balance nitrogen gas mixture (equivalent altitude of 23,300 ft or 7101 m) and the isocapnic hypoxia (IH) condition using a 7% oxygen (partial pressure 18.9 mmHg), 5% carbon dioxide (partial pressure 13.5 mmHg) with balance nitrogen gas mixture (equivalent altitude of 25,900 ft or 7894 m). Normoxia was achieved by providing room air through the mask inhalation hose.

All oculometric data were recorded during 1 min of mental workload challenge, which consisted of performing the K-D test. The Baseline oculometric data were collected under normoxia. As shown in **Fig. 2**, after the baseline recording, the oculometric data were collected under HH and then under IH conditions after 3 min of breathing the respective gases. The administration of the gas mixtures was double-blinded. A washout period (6 min) of normoxia was provided between the two hypoxic gas mixtures to avoid possible lingering after-effects of the test gas mixtures. After each experimental condition, subjects breathed 3 min of room air to collect Post-Baseline eye-tracking under normoxic conditions. The S_pO_2 and $P_{ET}CO_2$ data were monitored throughout the experiments. At the end of the experiment, each subject was verbally queried regarding the presence of common hypoxic symptoms (such as lightheadedness, dizziness, drowsiness, warmth, paresthesia, increased breathing, loss of concentration, headache, tachycardia, blurred vision, etc.).

Statistical Analysis

The analysis of eye movements during the K-D test under the three different breathing conditions (HH, IH, and normoxia) focused on four separate categories: blinks, pupillary dynamics, fixations, and saccades. The raw scan-paths shown in Fig. 1 were used to extract measured features related to fixations and saccades. The measures related to fixations are average fixation time-length, average fixation size, fixation count, total fixating time, and fluctuation in fixation time length. The measures related to saccades are average saccadic length, total saccadic time, saccade count, saccadic amplitude, and saccadic velocity. The blinks were recorded from the loss of scan-path data when the eye-tracking device was unable to track the eyes due to eyelid closure. The measures that were extracted related to blinking were, therefore, exclusively time-related, i.e., blink rate, blink duration, and interblink duration. The measures related to pupillary dynamics are pupil diameter and pupil size fluctuation. Repeated measures ANOVA (rmANOVA) on the oculometric features was used to assess the differences in mean values across the three different breathing conditions.

RESULTS

All 25 subjects participating in the study completed the entire experimental sequence, including recordings in Baseline normoxia, HH, IH, and during the two Post-Baseline normoxia periods which followed after each hypoxic recording. For technical reasons, the scan-paths of six subjects were not as clearly recorded or stable during some of the conditions. As a result, we excluded scan-path data from these subjects in analyses related to fixations and saccades. No severe hypoxic symptoms were reported by any subject. Only mild symptoms were volunteered, including light-headedness (4 subjects), dizziness (4 subjects), increased rate and/or depth of breathing (2 subjects), loss of concentration (2 subjects), warmth (1 subject), lip paresthesia (1 subject), and blurred vision (1 subject).

S_pO_2 levels decreased from 98 ± 0.9% in the Baseline condition to 75.8 ± 8.3% after inhaling 3 min of HH gas mixture and to 81.1 ± 5.5% after the IH gas mixture (**Fig. 3A**). All subjects completely recovered to their normal S_pO_2 (98 ± 0.8%) levels following both hypoxic conditions. Every subject returned to the S_pO_2 level they had originally registered during Post-Baseline normoxic testing. $P_{ET}CO_2$ levels decreased from 36.2 ± 5.5 mmHg in the Baseline condition to 30.7 ± 3.2 mmHg after inhaling 3 min of HH gas mixture and increased to 42.4 ± 4.1 mmHg after the IH gas mixture (**Fig. 3B**). Respiratory rate in the Baseline condition (12.4 ± 5.7 breaths/min) was not significantly altered after inhaling 3 min of the HH gas mixture (12.1 ± 3.5 breaths/min) and after the IH gas mixture (12.6 ± 3.4 breaths/min).

K-D test completion time increased from 46.3 ± 10.4 s in the Baseline condition to 54.5 ± 12.4 s [$F(1,48) = 6.23$, $P = 0.016$, one-way ANOVA] after inhaling 3 min of the HH gas mixture and to 53.9 ± 14.5 s [$F(1,48) = 4.42$, $P = 0.04$, one-way ANOVA] after the IH gas mixture. K-D test errors in the Baseline condition were 0.4 ± 0.8 errors per person, which significantly increased to 1.6 ± 1.5 errors in the HH condition [$F(1,48) = 11.37$, $P = 0.0015$, one-way ANOVA]. Interestingly, no significant increase in errors was observed in the IH condition [0.3 ± 0.9 errors, $F(1,48) = 0.25$, $P = 0.62$, one-way ANOVA] compared to the Baseline.

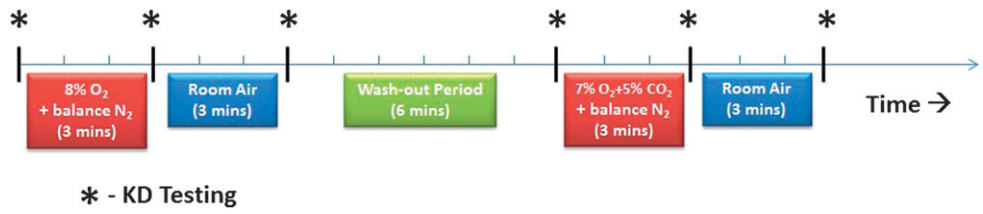

Fig. 2. Workflow of the entire experiment.

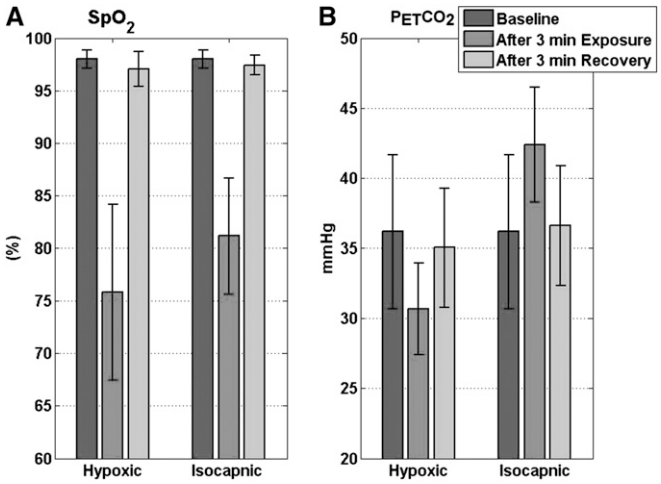

Fig. 3. Arterial oxygen saturation (S_pO_2) and end-tidal carbon dioxide ($P_{ET}CO_2$) levels during Baseline, after 3 min exposure to the hypoxic hypoxia (HH) and isocapnic hypoxia (IH) conditions, and after 3 min of recovery.

Eye Movement Data

Tables I and II provide the mean values of eye tracking measures in all four categories during the mental workload under the Baseline, HH, and IH conditions across all subjects. The hypoxic conditions were associated with a statistically significant effect on blink rate, interblink time interval, pupil size fluctuation, average fixation time length, average fixation size, fluctuation in fixation time-length, and total saccadic time compared with baseline values. **Table III** shows the percentage of tested subjects who had an increase, decrease, or no change from their baseline eye tracking features in the HH condition. IH was not associated with any changes from baseline.

Blink Parameters

Blink rate during the mental workload challenge under the HH condition was significantly higher as compared to the Baseline condition with a percentage change of +100%, i.e., twice (Table I), with high intersubject variability. The results from repeated measure ANOVA showed a significant effect of HH on blink rate [$F(1,24) = 5.69$, $P = 0.025$]. The blink rate under the IH condition (Table I) was also significantly higher than that under the Baseline condition [18.2 vs. 11.9, with 92.8% change; $F(1,24) = 5.4$, $P = 0.03$]. It was, however, also lower than under the HH condition even though the percentage of oxygen was less in the former gas mixture (7% vs. 8% oxygen).

Fig. 4A shows boxplots of raw data for blink rate in the baseline, hypoxic, and post-hypoxic conditions. In more detail, **Fig. 4B** shows the percentage change in the blink rate from Baseline to Post-Baseline conditions after IH was significantly lower compared to the Post-Baseline condition after HH (23.6% vs. 76.3%). This indicates that blink rate after IH recovered to normal baseline levels at a faster rate compared to that during the HH condition. The average interblink intervals under both hypoxic conditions were reduced to less than 5.5 s from the 7.7 s recorded under the Baseline condition, which constitutes a significant difference by repeated measures ANOVA [$F(2,48) = 4$, $P = 0.02$].

Since the repeated measures ANOVA did not show a statistically significant effect of hypoxic conditions on blink duration under the conditions of these experiments, we analyzed the blink duration distribution in three different categories which were labeled short blink (60-100 ms), medium blink (100-170 ms), and long blink (170-300 ms). **Fig. 5** shows the distribution of the total blink count recorded during the entire experiment as well as the relative percentages in each of the three blink duration categories during the Baseline, HH, and IH conditions. Under HH, the total percentage of short blinks was significantly greater than under the Baseline or IH conditions. Regardless of the difference between total blink counts, the blink duration distribution under IH was similar to the distribution under Baseline normoxia.

Pupillary Dynamics

Fluctuation in pupil diameter was significantly increased under HH (Table I) as compared to Baseline values ($P < 0.05$). There was, however, no significant change under IH (Table I) compared to Baseline data. **Fig. 6A** shows boxplots of raw data for pupil size fluctuation in the baseline, hypoxic, and post-hypoxic conditions. As further seen in **Fig. 6B**, the percentage change in the fluctuation in pupil diameter from the Baseline

TABLE I. COMPARISONS OF BLINK AND PUPILLARY DYNAMIC MEASUREMENTS BETWEEN BASELINE AND DIFFERENT HYPOXIC CONDITIONS DURING THE K-D TEST.

Eye Tracking Measures	Baseline (Normoxic)	Hypoxic Hypoxia	Isocapnic Hypoxia	rmANOVA
Blinks				
Blink Rate (blinks per minute)	11.9 ± 11.1 (6.3-17.5)	22.5 ± 28.2 (16.9-28.1)	18.2 ± 12.3 (12.6-23.8)	$F(2,48) = 3.53$, $P = 0.037$
Blink Duration (ms)	150.7 ± 78 (133.2-168.2)	122.7 ± 37.5 (105.2-140.2)	139.7 ± 42.4 (122.2-157.2)	$F(2,48) = 1.73$, $P = 0.1$
Interblink Time Interval (seconds)	7.65 ± 7.8 (6.56-8.75)	5.3 ± 6 (4.2-6.4)	5.7 ± 5.4 (4.6-6.8)	$F(2,48) = 4$, $P = 0.02$
Pupillary Dynamics				
Pupil Diameter (mm)	4 ± 0.5 (3.95-4.05)	4.1 ± 0.6 (4.05-4.15)	4 ± 0.5 (3.95-4.05)	$F(2,48) = 3.81$, $P = 0.03$
Pupil Size Fluctuation (mm)	0.12 ± 0.05 (0.11-0.13)	0.15 ± 0.05 (0.14-0.16)	0.11 ± 0.04 (0.1-0.12)	$F(2,48) = 4.7$, $P = 0.01$

Values are means ± SD, with 95% CI in parentheses.

TABLE II. COMPARISONS OF FIXATION AND SACCADIC FEATURES BETWEEN BASELINE AND DIFFERENT HYPOXIC CONDITIONS DURING THE K-D TEST.

Eye Tracking Measures	Baseline (Normoxic)	Hypoxic Hypoxia	Isocapnic Hypoxia	rmANOVA
Fixations				
Average Fixation Time (ms)	305 ± 75 (293-317)	320 ± 66 (308-332)	302 ± 72 (290-314)	$F(2,36) = 2.73$, $P = 0.07$
Average Fixation Size (inches)	3.1 ± 0.6 (3.0-3.2)	3.4 ± 0.8 (3.3-3.4)	3.2 ± 0.7 (3.1-3.3)	$F(2,36) = 4.01$, $P = 0.027$
Number of Fixations (#)	136 ± 23 (131-141)	139 ± 20 (134-144)	137 ± 20 (132-142)	$F(2,36) = 0.16$, $P = 0.8$
Total Fixating Time (second)	41.1 ± 10.1 (39-43.2)	44 ± 9.9 (41.9-46.1)	41.8 ± 13.5 (39.7-43.9)	$F(2,36) = 2.03$, $P = 0.1$
Fluctuation in Fixation length (ms)	138 ± 33.1 (128-148)	162.5 ± 42.6 (152.5-172.5)	149.8 ± 46.8 (139.8-159.8)	$F(2,36) = 6.07$, $P = 0.005$
Saccades				
Average Saccadic Length (inches)	1.92 ± 0.24 (1.89-1.95)	1.91 ± 0.24 (1.88-1.94)	1.94 ± 0.26 (1.91-1.97)	$F(2,36) = 0.9$, $P = 0.4$
Total Saccadic Time (seconds)	8.9 ± 4.6 (7.3-10.5)	10.8 ± 3.9 (9.2-12.4)	11.6 ± 6.7 (10-13.2)	$F(2,36) = 3.07$, $P = 0.05$
Number of Saccades (#)	274 ± 101 (244-304)	276 ± 79 (246-306)	297 ± 151 (267-327)	$F(2,36) = 0.71$, $P = 0.5$
Saccadic Amplitude (degrees)	4.3 ± 0.5 (3.1-5.5)	4.2 ± 0.5 (3-5.4)	4.35 ± 0.58 (3.15-5.55)	$F(2,36) = 0.91$, $P = 0.4$
Saccadic Velocity (degrees/second)	107.7 ± 13.5 (106.5-108.9)	106.9 ± 13.6 (105.7-108.1)	108.9 ± 14.6 (107.7-110.1)	$F(2,36) = 0.9$, $P = 0.4$

Values are means ± SD, with 95% CI in parentheses.

normoxic condition significantly increased under HH, much more than during IH (29% vs. 4.4%). After both hypoxic conditions, the fluctuation in pupil diameter returned to normal levels during mental workload testing with Post-Baseline normoxia.

Fixation and Saccades

We defined "total fixating time" as the cumulative time of all fixations during the performance of the entire test (i.e., reading three K-D test cards). There was an 8% increase in total fixating time from Baseline to the HH condition as compared to a 0.4% increase from Baseline to the IH condition (Table II). Although the difference of total fixating time between Baseline and HH was not statistically significant, an increase in total fixating time of 3 to 4 s could still be considered a marker of altered cognition (12). To investigate this further, we also defined "fixation time" as the testing time spent by each subject with eyes in fixation. The analysis of fixation time (Table II) showed a significant increase during HH as compared to Baseline normoxia [$F(1,18) = 11.9$, $P = 0.003$]. On the other hand, there was no statistically significant difference in the variation in fixation time between Baseline and IH. The average fixation time in HH was significantly higher than in IH [$F(1,18) = 5.4$, $P = 0.03$]. In addition, the average fixation size during HH was significantly larger than that during IH [$F(1,18) = 4.8$, $P = 0.04$] and larger than during the Baseline condition [$F(1,18) = 5.7$, $P = 0.03$]. Finally, there was no statistically significant difference between Baseline and IH in terms of values of average fixation time and fixation size.

Measures of saccadic function such as average saccadic length, total saccade count, saccadic amplitude, and saccadic velocity did not show any significant differences between Baseline values and those in either hypoxic condition. The mean values of all saccadic measures were, however, marginally higher during IH than during HH as opposed to the values during Baseline (see Table II). The total saccadic times under both hypoxic conditions were significantly increased compared with values under Baseline conditions ($P < 0.05$), but not different from each other.

TABLE III. PERCENTAGE OF TESTED SUBJECTS ($N = 25$) WHO HAD INCREASE, DECREASE, OR NO CHANGE FROM THEIR BASELINE EYE TRACKING FEATURES IN THE HYPOXIC HYPOXIA (HH) CONDITION.

Eye Tracking Features	Increase (%)	Decrease (%)	No Change (%)
Blink Rate	72	16	12
Pupil Size Fluctuation	68	24	8
Average Fixation Time	72	16	12
Fixation Size	56	24	20
Fluctuation in Fixation Length	72	28	0
Total Saccadic Time	72	24	4
Saccadic Velocity/ Amplitude	28	64	8

DISCUSSION

Eye movement metrics have recently been recognized as promising indicators for estimating changes in brain activity that reflect altered cognitive performance. Pupillary dynamics (29), eye movements (i.e., fixations and saccades) (15), and blink metrics (30) have all been linked to cognitive activity. Changes in these parameters have been reported in hypoxia and altitude acclimatization (6,26,29) and, therefore, might have a promising role in extreme and highly demanding environments such as aviation and mountaineering. In these settings detection of early signs of compromised performance associated with hypoxia is pivotal to mitigate the risk of accidents.

Even though eye movement metrics have been considered reliable measures of cognitive activity (16),

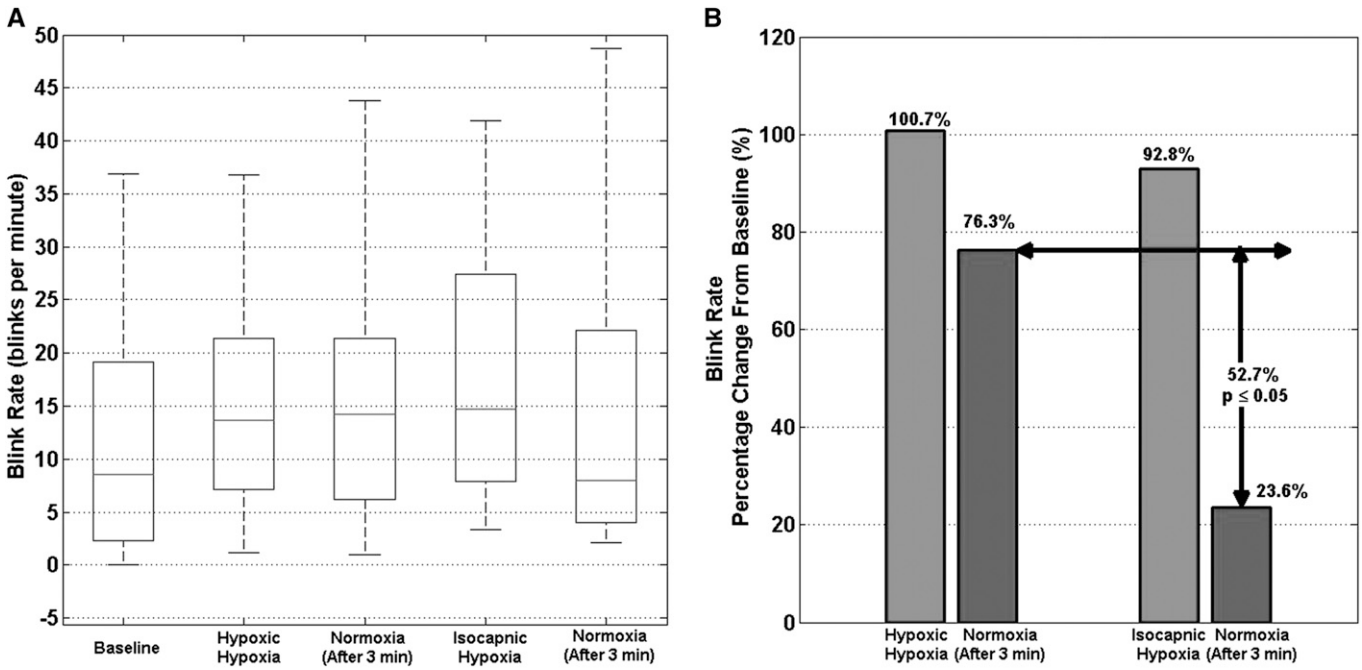

Fig. 4. A) Boxplots of raw data for blink rate in the Baseline, hypoxic, and post-hypoxic conditions. B) Percentage change in the blink rate from Baseline to hypoxic hypoxia (HH), isocapnic hypoxia (IH), and respective Post-Baseline values (Note: *P*-value is based on comparing raw values using repeated measures ANOVA).

quantitative measurements can be problematic due to varying levels of mental workload (22). In the present study subjects were tested under hypoxic and normoxic conditions using precisely the same level of cognitive workload. Furthermore, eye movement changes in hypoxia were normalized with respect to the corresponding normoxic eye movement changes in order to remove intersubject variability.

Changes in oculometrics at low altitudes have been previously reported (4,6); however, the present study is the first to report changes of multiple oculometric parameters through a noncontact computerized eye-tracking device in the early phase of an acute hypoxic exposure (> 6,096 m or 20,000 ft). This approach avoided the confounding variable of adaptive responses of the body (acclimatization). Moreover, in addition to the oxygen saturation data, which only estimates the oxygen tension, we also reported $P_{ET}CO_2$ as a measure of relative capnic status, a surrogate measure for cerebral perfusion and tissue oxygen delivery which can be correlated to observed cognitive changes.

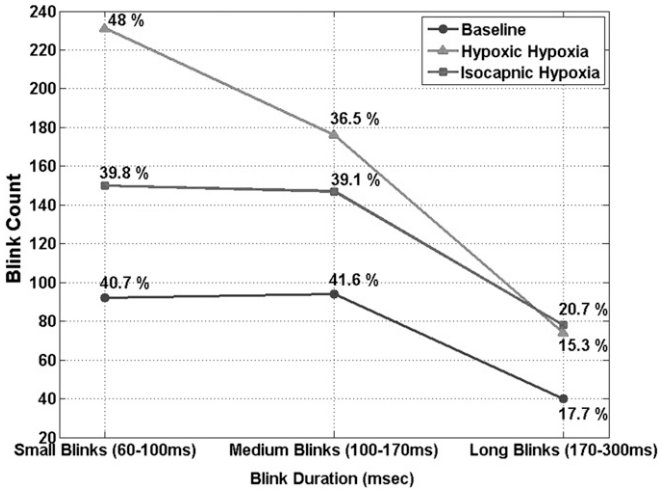

Fig. 5. Distribution of blink duration under Baseline, hypoxic hypoxia (HH), and isocapnic hypoxia (IH).

To further analyze the relationship between different levels of hypoxia and eye movement changes, we used two different hypoxic gas mixtures [HH (8% O_2) and IH (7% O_2 + 5% CO_2)]. In this way, we also evaluated whether the addition of carbon dioxide to the hypoxic gas mixture might improve oculometric performance based on the known cerebral vasodilatative and hence enhanced tissue oxygen delivery effects of adding carbon dioxide to the breathing gas mixture (11). The addition of carbon dioxide furthermore enhances oxygenation by improving pulmonary gas exchange at the level of the lung tissue, hence mildly improving oxygen saturation (2), as also observed in our data (S_pO_2 in HH 75.8 ± 8.3%; and 81.1 ± 5.5% in IH) (Fig. 3). The improvement in oxygen saturation mirrored cognitive performance, where K-D test errors in the IH condition were improved to 0.3 ± 0.9 errors per person (close to normoxia) from 1.6 ± 1.5 errors in the HH condition. Respiratory rate, however, in both hypoxic conditions was not different. We hypothesize that the acute exposure in this experiment led to a stress response for both hypoxic gases, overriding the expected CO_2/O_2 driven alterations in respiratory rate.

Oculometric parameters such as blink rate have been shown to increase during states of anxiety, visual fatigue, sleep deprivation, driving or flying, and during tasks requiring speech, memory, or mental challenge, but decrease during reading or viewing text on a video

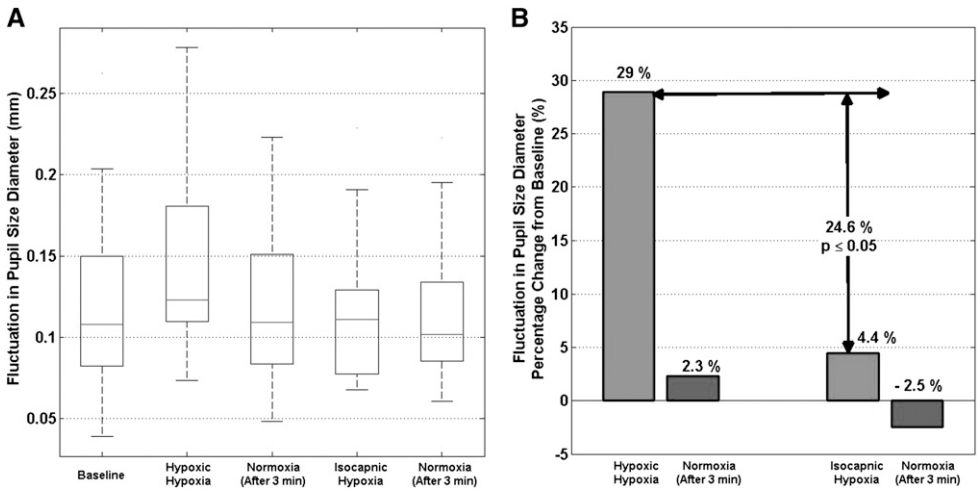

Fig. 6. A) Boxplots of raw data for pupil size fluctuation in the Baseline, hypoxic, and post-hypoxic conditions. B) Percentage change in the pupil size fluctuation from Baseline to hypoxic hypoxia (HH), isocapnic hypoxia (IH), and respective Post-Baselines (Note: *P*-value is based on comparing raw values using repeated measures ANOVA).

display (5,19). Data regarding the direct effects of hypoxia on blink metrics have not yet been reported. Our results show a significant increase in the blink rate in HH compared with Baseline values (100.7% change, $P = 0.03$). Blink rate was increased in 72% of the tested subjects, decreased in 16%, and there was no change in 12% (Table III). This evidence supports the hypothesis that hypoxia might activate a sympathetic autonomic response in the early phase of hypoxic exposure. An increased release of dopamine in hypoxia, as previously reported by Panjwani et al. (20), may be directly involved in the increase of blink rates. This neurotransmitter is well known to play a role in controlling blink activity (3). Blink rates in IH, however, did not show any further increases despite the fact that the percentage of oxygen was slightly lower in this gas mixture (7% vs. 8%). Moreover, a faster recovery of blink rate back to baseline levels was observed after IH (Fig. 4B). These results in aggregate might suggest a potential role of carbon dioxide in modulating the sympathetic response during hypoxia given the well-known benefit in improving brain perfusion in the face of oxygen deficiency (10). This is the result of the combined effect of the hypercapnic cerebral vasodilation and the rightward shift of the oxygen-hemoglobin dissociation curve (induced by the acidosis), which increases oxygen delivery to tissues (1,27). Moreover, acidosis reduces cellular respiration and oxygen consumption, which may further benefit the imbalance between supply and demand in hypoxic conditions (13).

Similar results have been obtained studying pupillary function, where results in the HH condition showed greater pupil size fluctuation as compared with the diameter range in IH (0.15 vs. 0.11 mm, $P = 0.01$). Pupil size fluctuation in HH was increased in 68% of the tested subjects, decreased in 24%, and there was no change in 8% (Table III). Our results confirm the observations of Thomson et al. (25) regarding pupillary diameter fluctuation due to the reciprocal balance between sympathetic and parasympathetic innervations of the iris when consciousness or alertness is reduced. Our results also suggest that, during HH, fluctuations in autonomic control of the pupil may provide a potential early indicator of hypoxia. On the other hand, fluctuations in pupil diameter during IH were similar to those observed under Baseline conditions. These results suggest that addition of carbon dioxide to the hypoxic mixture might have played a role in reducing pupil diameter fluctuations. However, future studies are needed to further assess the clinical relevance of our statistically significant findings in pupil size fluctuation.

Fixational eye movements have recently been recognized as relevant indicators of attention and cognitive engagement (17). As part of fixational movements, microsaccades contribute to maintenance of visibility during fixation by shifting the retinal image to counteract visual adaptation. Recent discoveries have established a link between microsaccades and cognitive processes such as attention and working memory (14). The significant increases in average fixation time and fixation size in HH found in the present study suggest that hypoxia might be the result of an impairment of fixation stability and an increase in microsaccades while reading numbers on the K-D test. Total fixation time in hypoxic conditions was increased in 72% of the tested subjects, decreased in 16%, and there was no change in 12% (Table III).

Our results support the previous findings of Martinez-Conde et al. (18), which reported the excessive eye movements responsible for blurring and unstable vision during fixation might account for the visual impairment frequently reported under hypoxic conditions. Descriptions on the impact of hypoxia on saccadic parameters are scarce in the literature. Cymerman et al. (6) were the first to measure saccadic velocity in 18 subjects exposed to hypobaric hypoxia (4300 m altitude) for 14 d. Initially saccadic velocity increased over 6 d, but then returned toward baseline levels, following similar changes in

Po_2, Pco_2, HR, oxygen saturation, and catecholamine levels. In the present study, neither saccadic velocity nor saccadic amplitude showed significant differences between Baseline and hypoxic conditions. There was, however, an increase in total saccadic time under both hypoxic conditions compared with the Baseline normoxic condition; this might relate to the instability of fixation and dysfunctional saccades during the hypoxic conditions. Total saccadic time in the hypoxic conditions were increased in 72% of the tested subjects, decreased in 24%, and there was no change in 4% (Table III).

While double-blinded, the lack of randomization of the gas order is an important limitation of this study. In summary, these results showed oculometric measures such as blink rate, pupil size fluctuation, average fixation size, and total saccadic time to be the most sensitive in detecting presymptomatic hypoxic changes. These unobtrusively measured oculometrics merit further study in a cockpit environment to detect early changes in aircrew. This data also highlights the potential role of carbon dioxide in favorably modulating physiological responses and performance under hypoxic conditions. Based on this pilot data, larger, randomized trials should be considered. These findings may encourage further investigation of the use of oculometric measures for the detection of cognitive performance decrements due to the influence of acute hypoxia. The notion of using noninvasive performance metrics closely tied to visual flying tasks such as scanning the horizon or the instrument panel deserves further investigation, especially as it pertains to the assessment of hypoxic reserve time and early warning.

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