Comparison of Different Hyperoxic Paradigms to Induce Vasoconstriction: Implications for the Investigation of Retinal Vascular Reactivity

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PURPOSE. To compare the impact of three different techniques used to induce hyperoxia on end-tidal CO2 (PETCO2). The relationship between change in PETCO2 and retinal hemodynamics was also assessed to determine the clinical research relevance of this parameter.

METHODS. The sample comprised 10 normal subjects (mean age, 25 years; range, 21–49 years). Each subject attended for three sessions. At each session, subjects initially breathed air followed by O2 only; O2 plus CO2, using a nonrebreathing circuit (with CO2 flow continually adjusted to negate drift of PETCO2); or air followed by O2, using a sequential rebreathing circuit. In addition, using a separate sample of eight normal subjects (mean age, 26.5 years; range, 24–36 years), a methodology that initially raised PETCO2 and then returned to homoeostatic levels was used to determine the impact, if any, of perturbation of PETCO2 on retinal hemodynamics.

RESULTS. The difference in group mean PETCO2 between baseline and elevated O2 breathing was significantly different (t-test, P = 0.0038) for O2-only administration with a nonrebreathing system. The sequential rebreathing technique resulted in a significantly lower difference (i.e., before and during hyperoxia) of individual PETCO2 (t-test, P = 0.0317). The PETCO2 perturbation resulted in a significant (P < 0.005) change of retinal arteriolar diameter, blood velocity, and blood flow.

CONCLUSIONS. The sequential rebreathing technique resulted in a reduced variability of PETCO2. A relatively modest change in PETCO2 resulted in a significant change in retinal hemodynamics. Rigorous control of PETCO2 is necessary to attain standardized, reproducible hyperoxic stimuli for the assessment of retinal vascular reactivity. (Invest Ophthalmol Vis Sci. 2004;45:3207–3212) DOI:10.1167/iovs.03-1223

Administration of oxygen (O2) has been used as a stimulus to provoke retinal vascular reactivity. Vasoconstriction of retinal vessels1,2 and the resulting reduction of retinal blood flow3–16 has been demonstrated with a variety of measurement techniques, including laser Doppler and blue-field entoptic phenomena. Retinal blood flow varies inversely with the partial pressure of arterial oxygen (PO2) to maintain retinal oxygenation at a relatively constant level16; however, retinal blood flow also varies directly with the partial pressure of arterial carbon dioxide (PACO2).1,2 The change of end-tidal CO2 concentration (PETCO2; the maximum concentration of CO2 during each expiration) reflects the change in arterial PCO2.1,2 Indeed, CO2 is thought to represent a more potent vasoactive agent than O2.7 Change of retinal perfusion, measured using laser Doppler blood flow techniques, induced by perturbation of O2 or CO2 can be used to provide a measure of the magnitude of retinal vascular reactivity.

All previously published retinal vascular reactivity studies have used gas delivery systems that comprise a reservoir bag and one-way valves to essentially negate the mixing of inspired and expired gases (i.e., a nonrebreathing system). Hyperoxia typically stimulates hyperventilation (faster or deeper respiration); however, this results in an uncontrolled and variable reduction of PCO2.18,19 Any reduction of PETCO2 produces an exaggerated vasoconstrictive effect on retinal vasculature. The vasoconstrictive effect previously attributed to O2 when administered by nonrebreathing circuits probably represents the combined effect of elevated PO2 and reduced PETCO2. Harris et al.,16,20,21 Roff et al.,14 and Chung et al.15 have recognized this potential artifact and have attempted to correct for the reduction in PCO2 during hyperoxia by adding CO2 to the inspired gases of the nonrebreathing system.14,16,20,21 The maintenance of homeostatic PETCO2 is termed isocapnia. Another method to prevent reduction of PCO2 involves the use of a sequential rebreathing circuit that provides a feedback loop to compensate for any hyperventilation induced reduction in PCO2.22 This system has the advantage that it passively adjusts the inspired CO2 to the minute ventilation to stabilize PETCO2.

The magnitude of change and the variability of PETCO2 should be quantified and compared across the various techniques used to induce hyperoxia. Three different techniques were compared: administration of O2 only with a nonrebreathing system; O2 with added CO2, with a nonrebreathing system (with CO2 flow continually adjusted to negate “drift” of PETCO2); and O2 using a sequential rebreathing system (with O2 flow set equal to the subjects’ minute ventilation). In addition, the relationship between change in PETCO2 and retinal blood flow was assessed to determine the clinical research relevance of this parameter.

MATERIALS AND METHODS

Sample

The study received approval by the University of Waterloo Office of Research Ethics. Informed consent was obtained from each subject after explanation of the nature and possible consequences of the study, according to the tenets of the Declaration of Helsinki. The sample...
comprised four men and six women of average age 25 years (range, 21–49 years). To determine the relationship between P$_{ET}$CO$_2$ and retinal blood flow, a second sample of six men and two women of average age 26.5 years (range, 24–36 years) was subsequently recruited. Subjects with any cardiovascular or respiratory disorders were excluded from the study.

**Procedures**

Each subject attended three sessions of approximately 30 minutes each. The group mean number of days between each of the three sessions was 11 days. At each session, subjects initially breathed air followed by O$_2$ only, or O$_2$ plus CO$_2$ using a nonrebreathing system (Fig. 1), or compressed air followed by O$_2$ using a sequential rebreathing system (Fig. 2). Each gas condition (air or O$_2$) was administered for 15 minutes. The nonrebreathing sample comprised a silicone mouthpiece and two low-resistance one-way valves connected to the gas supply by a reservoir bag. The sequential rebreathing system comprised fresh gas and rebreathed gas reservoirs that were interconnected by two one-way valves and a single peep valve. It was assembled by adding a gas reservoir to the expiratory port of a commercial three-valve oxygen-delivery system (Hi-Ox<sup>®</sup>; Viasys Healthcare, Yorba Linda, CA). For the purpose of this study, a silicone mouthpiece was attached to the sequential rebreathing system, which in turn was connected to the gas supply. For both systems, flow from the gas tanks was controlled using standard rotometers as flowmeters. O$_2$ and CO$_2$ were mixed in a baffled container before being administered to the subject through the nonrebreathing circuit.

Each subject was seated for 5 minutes before commencing the study. In every situation, an initial air-breathing period was used to allow stabilization of baseline breathing parameters (e.g., respiration rate). For the O$_2$ plus CO$_2$ with a nonrebreathing system (Fig. 1), CO$_2$ flow was continually adjusted to negate drift of P$_{ET}$CO$_2$. For the O$_2$ using a sequential rebreathing system (Fig. 2), O$_2$ flow was set equal to the subjects' minute ventilation (determined while breathing air).
To determine the impact, if any, of perturbation of P\textsubscript{ET}CO\textsubscript{2} on retinal blood flow, the sequential rebreathing system was used to manipulate P\textsubscript{ET}CO\textsubscript{2} while retinal blood flow was quantified with a laser blood flowmeter (CLBF 100; Canon, Tokyo, Japan). A steady state perturbation of P\textsubscript{ET}CO\textsubscript{2} was produced because the time between P\textsubscript{ET}CO\textsubscript{2} fluctuation and its impact on retinal hemodynamics is unknown and because the CLBF does not provide a continuous measurement of retinal blood flow. A methodology that initially raised P\textsubscript{ET}CO\textsubscript{2} and then returned to homeostatic levels was used. After stabilization of cardiovascular and respiratory parameters, air flow delivered to the subject through the sequential rebreathing system was reduced to elevate P\textsubscript{ET}CO\textsubscript{2} by approximately 5 mmHg (i.e., the volunteers were compelled to rebreathe). At this point, a minimum of 10 CLBF readings was acquired. Air flow was subsequently returned to baseline levels, and a further six CLBF readings were acquired.

**Data Acquisition and Analysis**

Tidal gas concentrations were continuously sampled from the mouthpiece using a rapid-response critical care gas analyzer (Cardiocap 5; Datex-Ohmeda, Louisville, CO). In addition, hemoglobin oxygen saturation through pulse oximetry and respiratory and pulse rate were also continuously recorded. All data outputs were downloaded to an electronic data acquisition system (S5 Collect; Datex-Ohmeda). Data were analyzed using box plots that depicted the median, top 25th and bottom 75th percentiles (SD) and outliers of inspired- and end-tidal gas concentrations. Data points lying outside the top 25th or lower 75th percentiles were excluded from the analysis, because all these values were found to be erroneous—that is, these points resulted from inappropriate interpretation of tidal waveforms by the gas monitor.

Group mean inspired and expired O\textsubscript{2} (FiO\textsubscript{2} and P\textsubscript{ET}O\textsubscript{2}, respectively), inspired and expired CO\textsubscript{2} (FiCO\textsubscript{2} and P\textsubscript{ET}CO\textsubscript{2}, respectively), and respiration rates (RR) as a function of delivery system are shown in Table 1. The group mean FiO\textsubscript{2} was greater than 90% for all techniques. For O\textsubscript{2} breathing only, with a nonrebreathing system, group mean P\textsubscript{ET}CO\textsubscript{2} reduced from 5.32% ± 0.18% at baseline to 5.06% ± 0.18% during hyperoxia (Fig. 3A). For O\textsubscript{2} with added CO\textsubscript{2} using a nonrebreathing system, group mean P\textsubscript{ET}CO\textsubscript{2} was 5.34% ± 0.16% at baseline and 5.29% ± 0.17% during hyperoxia (Fig. 3B). For a sequential rebreathing system, group mean P\textsubscript{ET}CO\textsubscript{2} was 5.13% ± 0.15% at baseline and 5.07% ± 0.13% during hyperoxia (Fig. 3C). The difference in group mean P\textsubscript{ET}CO\textsubscript{2} between baseline and hyperoxia was significantly different for O\textsubscript{2} only and a nonrebreathing system (t-test, P = 0.0038) but not for the other two techniques.

The sequential rebreathing technique resulted in a significantly lower difference (i.e., a smaller difference between baseline and during hyperoxia) of individual P\textsubscript{ET}CO\textsubscript{2} (as reflected in the standard deviations, Table 2) than either of the other two techniques (t-test, P = 0.0008 and P = 0.0317 for O\textsubscript{2} only and O\textsubscript{2} with added CO\textsubscript{2}, respectively).

The group mean difference between the elevated (group mean 5.69% ± 0.44%) and homeostatic (group mean 5.03% ± 0.59%) P\textsubscript{ET}CO\textsubscript{2} conditions was 0.66% ± 0.21%—that is, a 5.00 ± 1.58 mm Hg change. This perturbation of P\textsubscript{ET}CO\textsubscript{2} resulted in a significant reduction (i.e., in response to a lowering of P\textsubscript{ET}CO\textsubscript{2}) in retinal arteriolar diameter, blood velocity and blood flow of 6.18 μm (P < 0.0050), 6.68 mm/sec (P = 0.0005), and 5.04 ul/min (P < 0.0005), respectively (Fig. 4).

**Discussion**

Elevating Po\textsubscript{2} by simply raising the FiO\textsubscript{2} without taking any measures to control PCO\textsubscript{2} resulted in a significant reduction from baseline in mean P\textsubscript{ET}CO\textsubscript{2}. This group mean reduction was ameliorated with coadministration of O\textsubscript{2} and CO\textsubscript{2} or the sequential rebreathing technique. Of the latter two methods, the sequential rebreathing technique had a significantly smaller variability in individual P\textsubscript{ET}CO\textsubscript{2} measurements.

Nonrebreathing techniques involve the administration of gas using a reservoir bag through a one-way "demand" valve—that is, a valve that opens at the onset of each inspiration. Expired gas leaves the system through a second one-way valve. Riva et al.\textsuperscript{4} were the first to describe retinal vascular effects using 100% O\textsubscript{2} and laser Doppler velocimetry. In terms of vision-science–based studies, Harris et al.\textsuperscript{16,20,21} were the first to consider the potential confounding factor of change in P\textsubscript{ET}CO\textsubscript{2} during hyperoxia by coadministering O\textsubscript{2} and CO\textsubscript{2} with a nonrebreathing system. Roff et al.\textsuperscript{14} and Chung et al.\textsuperscript{15} also used this technique in their ocular blood flow studies. These studies did not report the magnitude of individual variability of P\textsubscript{ET}CO\textsubscript{2}. This study demonstrates that the maintenance of homeostatic P\textsubscript{ET}CO\textsubscript{2} levels using nonrebreathing techniques apply to groups as a whole, but are less reliable for individual subjects.

**Results**

The group mean difference of P\textsubscript{ET}CO\textsubscript{2} (and SD) between baseline and elevated O\textsubscript{2} breathing for each of the three techniques is shown in Table 2. Figure 3 shows P\textsubscript{ET}CO\textsubscript{2} for each individual at baseline (i.e., air) and during hyperoxia for each of the three techniques.

**Table 1. Group Mean and SD of Inspired O\textsubscript{2}, Expired O\textsubscript{2}, Inspired CO\textsubscript{2}, End-tidal CO\textsubscript{2}, and Heart Rate as a Function of Technique**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Air (O\textsubscript{2})</th>
<th>O\textsubscript{2} (O\textsubscript{2}-air)</th>
<th>Total O\textsubscript{2}</th>
<th>Air (O\textsubscript{2})</th>
<th>O\textsubscript{2} (O\textsubscript{2}-air)</th>
<th>Total O\textsubscript{2}</th>
<th>Heart Rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure O\textsubscript{2} 20.33</td>
<td>19.91</td>
<td>19.50</td>
<td>20.00</td>
<td>0.17</td>
<td>0.18</td>
<td>0.17</td>
<td>72.96</td>
</tr>
<tr>
<td>SD 0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>O\textsubscript{2}+CO\textsubscript{2} 20.28</td>
<td>19.87</td>
<td>19.46</td>
<td>19.90</td>
<td>0.17</td>
<td>0.18</td>
<td>0.17</td>
<td>72.96</td>
</tr>
<tr>
<td>SD 0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>O\textsubscript{2} (SRB) 19.91</td>
<td>19.50</td>
<td>19.09</td>
<td>19.63</td>
<td>0.17</td>
<td>0.18</td>
<td>0.17</td>
<td>72.96</td>
</tr>
<tr>
<td>SD 0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Table 2. Group Mean Difference in P\textsubscript{ET}CO\textsubscript{2} (%) between Baseline and Oxygen Breathing Using Three Different Techniques**

<table>
<thead>
<tr>
<th>Pure O\textsubscript{2}</th>
<th>O\textsubscript{2}+CO\textsubscript{2}</th>
<th>SRB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group mean difference</td>
<td>−0.21</td>
<td>−0.06</td>
</tr>
<tr>
<td>(O\textsubscript{2}-air)</td>
<td>0.24</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Furthermore, the link between change in P\textsubscript{ET}CO\textsubscript{2} and retinal hemodynamics (namely retinal arteriolar diameter, blood velocity, and blood flow) has been demonstrated. The group mean change of P\textsubscript{ET}CO\textsubscript{2} of 0.66% produced a group mean 27% change in retinal blood flow (i.e., a 2-mmHg drift in P\textsubscript{ET}CO\textsubscript{2}) that invariably occurs with nonrebreathing techniques, resulting in a 10% to 12% artifactual change in blood flow. This emphasizes the importance of using breathing circuits that facilitate control of P\textsubscript{ET}CO\textsubscript{2} measurements during administration of elevated O\textsubscript{2}.

An alternative method of preventing reduction of P\textsubscript{ET}CO\textsubscript{2} with increases in ventilation (as induced by exposing subjects to O\textsubscript{2}) is to increase the dead space of the circuit so that rebreathing occurs. Adding circuit dead space may not limit the reduction of P\textsubscript{ET}CO\textsubscript{2} with hyperventilation, as spontaneously breathing subjects (as opposed to those being mechanically ventilated) will overcome the effects of rebreathing by increasing respiratory volume. The sequential rebreathing method developed by Sommer et al.\textsuperscript{22} and Banzett et al.\textsuperscript{23} used in this study, passively matches the inhaled CO\textsubscript{2} to increases in minute ventilation thereby preventing the expected reduction in P\textsubscript{CO}\textsubscript{2}. The sequential rebreathing technique is effective irrespective of the pattern of breathing. Compared to a nonrebreathing system and adding CO\textsubscript{2} to inspired gas, this system has the advantage of avoiding the risk of raising P\textsubscript{ET}CO\textsubscript{2} and consequently eliciting subject discomfort. The flow of fresh gas (air or O\textsubscript{2} not containing CO\textsubscript{2}) is set to just match the patient’s

![Figure 3](image-url)  
**Figure 3.** Change in end-tidal CO\textsubscript{2} concentration for each individual using (A) pure O\textsubscript{2} delivered by a nonrebreathing system, (B) O\textsubscript{2} with added CO\textsubscript{2} delivered through a nonrebreathing system, and (C) O\textsubscript{2} delivered through a sequential rebreathing (SRB) system.

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![Figure 4](image-url)  
**Figure 4.** Change in retinal (A) arteriolar diameter, (B) blood velocity, and (C) blood flow induced by a change in end-tidal CO\textsubscript{2} concentration. The group mean difference between the elevated (group mean 5.69% ± 0.44%) and homeostatic (group mean 5.03% ± 0.59%) P\textsubscript{ET}CO\textsubscript{2} conditions was 0.66% ± 0.21%. Error bars, SD.
Hyperoxia-Induced Vasoconstriction

Using the coadministration of O\textsubscript{2} and CO\textsubscript{2}. In contrast, the sequential rebreathing technique was shown to allow the administration of elevated O\textsubscript{2} levels without a reduction in P\textsubscript{ET}CO\textsubscript{2} and, importantly, reduced variability of P\textsubscript{ET}CO\textsubscript{2} measurements when compared with the nonrebreathing techniques. Steady state manipulation of P\textsubscript{ET}CO\textsubscript{2} unequivocally demonstrated that relatively modest change in P\textsubscript{ET}CO\textsubscript{2} resulted in significant change of retinal hemodynamics.

CONCLUSIONS

Published retinal vascular reactivity studies have used a non-standardized hyperoxic stimulus without control of P\textsubscript{ET}CO\textsubscript{2}, making the results difficult to interpret. Compounded vasoconstrictive effects occur when no control for the reduction of systemic PCO\textsubscript{2} levels is made. In addition, blood flow measurements taken under these conditions may exhibit exaggerated variability due to continuous alterations in PCO\textsubscript{2}.

Rigorous control of P\textsubscript{ET}CO\textsubscript{2} using a modified commercially available sequential rebreathing circuit will allow the establishment of a standardized, reproducible hyperoxic stimulus for the investigation of vascular reactivity of the human retina.

References


